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(57) Abstract

Peptides having at least seven amino acids, preferably up to 20 amino acids, and most preferably 7 to 9 amino acids, comprising an amino acid sequence which corresponds to positions 180-186 of the *Mycobacterium tuberculosis* protein hsp 65 having the formula TFGLQLE, but differing therefrom by one to three amino acid substitutions in the positions 181-183, are disclosed. These peptides inhibit antigen recognition by T lymphocytes, such as recognition of hsp 65, and are capable of protecting a subject from an immune-related disease, such as autoimmune arthritis and to prevent rejection of a transplanted organ or tissue. Single amino acid substitutions, in particular with alanine, at positions corresponding to hsp 65 positions 183 and 181 are preferred. T cell lines specific for the above peptides and their use in treating or preventing immune-related disease is also disclosed.

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INHIBITOR OF LYMPHOCYTE RESPONSE AND IMMUNE-RELATED DISEASE

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention in the field of immunology and medicine relates to peptides which mimic a natural peptide and compete with the heat shock protein, hsp 65, or peptides derived therefrom for recognition by and activation of T lymphocytes. The peptides of the invention inhibit T lymphocyte activation and proliferative responses, and can protect a subject from immune reactions and immune-related disease including autoimmunity and graft rejection.

DESCRIPTION OF THE BACKGROUND ART

A. Autoimmunity and Arthritic Disease

Autoimmunity is thought to be based in part on the similarity in structure between a foreign molecule or antigen, and a molecular structure belonging to the organism ("self").

The list of autoimmune diseases is both long and disturbing. It includes, for example, multiple sclerosis, myasthenia gravis, rheumatoid arthritis, and systemic lupus erythematosus. In all of these diseases, the immune response is potent and specific. The problem is that this immune response is directed at some essential component of the body.

Millions of people are afflicted with chronic forms of arthritis which are thought to involve autoimmunity to constituents of the joints of connecting tissues of the body. These conditions include rheumatoid arthritis, ankylosing spondylitis, Reiter's syndrome, and other forms of reactive arthritis. The etiology of these diseases is not known, but previous infection with a variety of microbes seems to act as an inciting circumstance in genetically susceptible individuals. Patients with rheumatoid arthritis may show unusual reactivity to mycobacterial antigens, and immunization mycobacteria was found to lead to arthritis in 15 of 150 individuals.

B. Experimental Arthritis

Adjuvant arthritis is an experimental model of human arthritis inducible by immunizing susceptible strains of rats to Mycobacteria. The disease which develops about twelve days after immunization has many of the features of rheumatoid arthritis, and, indeed, adjuvant arthritis has been considered to be a model for rheumatoid arthritis. Rats immunized with adjuvant containing mycobacterial antigens to produce arthritis contain T lymphocytes specific for mycobacterial antigens. Indeed, long term T cell lines and clones have been isolated from rats so immunized; these T cell were able to induce and modulate the arthritic disease in vivo (Holoshitz, Science, 1983, 219:56; Holoshitz, J. et al., J. Clin. Invest., 1984, 73:211). For example, the T cell clone, designated A2b, was shown to induce arthritis in irradiated Lewis rats (Holoshitz, J. et al., J. Clin. Invest., 1984, 73:211). In contrast, a different T cell clone, designated A2c, acted as a suppressor-inducer T cell and counteracted the arthritogenic process. Rats treated with A2c cells acquired resistance to adjuvant arthritis (Cohen, I.R. et al., Arthritis Rheum., 1985, 29:841; Cohen, I.R. Immun. Rev., 1986, 94:5).

A first clue to the antigenic basis of adjuvant arthritis was obtained when it was found that the arthritogenic T cell clone, A2b, responded not only to mycobacteria, the immunogen which originally induced these cells, but also to crude cartilage extracts and those enriched in the proteoglycan core protein (Van Eden, W. et al., Proc. Natl. Acad. Sci. (USA), 1985, 82:5117). The minimal antigenic structure, or epitope, responsible for this mimicry between a cartilage-associated self structure and mycobacteria, recognized by both of the above T cell clones, is the amino acid sequence from residue 180 to 188 of the mycobacterial 65 kD heat-shock protein (HSP) (Van Eden, W. et al., Nature 1988, 331:171).

C. Heat Shock Proteins

Heat shock proteins (HSPs) are present in every type of cell examined, and are among the most conserved proteins known in phylogeny in both structure and function (Sette, A.

et al., Nature, 1986, 324:260; Shinnick, T.M., J. of Bacteriol., 1987, 169:1080; Kauffman, S.H.E., Immunol. Today 11:129 (1990)). HSPs can be divided into four major families, with members of molecular mass of about 90, 70, 60 and 10-30 kDa. Several 65 kD HSPs have been sequenced now (Shinnick, T.M., J. Bacteriol, 1987, 169:1080; Thole, J.E.R. et al., Microb. Pathogen, 1988, 4:71; Mehra, V. et al., Proc. Nat'l Acad. Sci. (USA), 1986, 83:7013; Hemmingson, S. et al., Nature, 1988, 333:330; Vodkin, H.M. et al., J. Bacteriol, 1988, 170:1227; Reading, D.S. et al., Nature, 1989, 337:655; Jindal, S. et al., Mol. and Cell. Biol., 1989, 9:2279). See Table 1, below for partial sequences.

HSPs serve as important antigens of infectious agents, and possibly of transformed cells. During infection, HSPs are induced in both microorganisms and host phagocytes. The dominant antigens of intracellular pathogenic microorganisms are HSPs known as hsp 65 (the human representative of the hsp 60 family of molecules) and hsp 70. Due to their extreme conservation, it is not surprising that HSPs bear a relationship with autoimmune diseases. Cloned "autoreactive" T cells have been obtained from normal donors that are reactive to human hsp 65 and hsp 70.

D. Immunity to Heat Shock Proteins in Arthritis

Adjuvant arthritis can be induced using M. tuberculosis in oil in Lewis rats and, to a lesser extent, in Fisher and BN rats. The expression of the mammalian homolog HSP is selectively enhanced in affected joint tissue of rats with adjuvant arthritis and patients with rheumatoid arthritis (Karlsson-Parra, A.K. et al., Scand. J. Immunol. 31:283-288 (1990)).

The inventors and their colleagues reported that the minimal stimulatory sequence for two arthritis-specific T lymphocyte clones, A2b and A2c, was defined as the 180-187 amino acids sequence of the mycobacterial hsp 65 (Van der Zee, R. et al., Eur. J. Immunol. 19:43 (1989)). Peptides having any amino acid replacement at positions 187 and 188 fully stimulated both T cell clones, indicating that these positions did not affect the antigenic nature of the 180-186

heptapeptide. It was thought that mimicry occurs with an hsp 65 epitope of a proteoglycan-associated HSP or an HSP expressed in synovial tissues.

Induction of arthritis in the Lewis rat is associated with T cell reactivity to the hsp 65 180-188 nonapeptide. However, two different inbred rat strains, Fisher and BN, fail to develop such a response. In a related experimental model in which arthritis is induced in Fisher rats by administering streptococcal cell walls (SCW), T cell reactivity to both SCW fragments and mycobacterial hsp 65 was absent, and no arthritis developed (Van den Broek, M.F. et al., J. Exp. Med. 170:449 (1989)).

A refractory state can be "artificially" induced in the Lewis rat adjuvant arthritis model by prior treatment with soluble mycobacterial hsp 65, which also down-regulates responses to the nonapeptide; this treatment also prevents SCW-induced arthritis and partially inhibits collagen-induced arthritis. There appears to be some specificity to the induced unresponsiveness

In human arthritis, including juvenile rheumatoid arthritis (JRA), T cell responses to mycobacterial hsp 65 are elevated in the synovial fluid, compared with blood; T cells from a few JRA patients with elevated antibody responses to hsp 65 responded to the 180-188 nonapeptide (Lydard, P.M. et al., Immunol. Today 11:228 (1990)). Thus, much of the specific response in JRA appears to be directed towards non-conserved regions of the HSP, since the mycobacterial hsp 65 180-188 nonapeptide is not contained within the mammalian sequence.

Synovial fluid-derived T cell clones induced by mycobacterial hsp 65 from a self-limiting reactive arthritis patient reacted to non-conserved N-terminal peptides of the antigen (Gaston, J.S.H. et al., J. Immunol. 143:2494-2500 (1990)). In Yersinia arthritis, 1 in 120 clones reacted with a shared (conserved) epitope, common to human and mycobacterial hsp 65 (see Lydard et al., supra).

It appears to be a paradox that human T cells with specificity for conserved HSP epitopes are present in normal

individuals given the dominance of HSPs as microbial antigens. It is postulated that lymphocytes forming a natural network carry receptors directed towards a limited set of dominant self-antigens, for example HSPs. It would be of great benefit to be able to modulate this network by vaccination with HSP peptides.

Stanford et al. (European Patent Publication EP 181364 (5/21/86)) discloses aqueous acetone soluble and insoluble fractions of certain mycobacteria, such as Mycobacterium H-37, M. kansasii and M. vaccae. The soluble fraction of H-37 was found to provoke an immune response leading to resistance to adjuvant arthritis whereas the insoluble fraction seemed to be responsible for the induction of the disease. M. vaccae was shown to be substantially free of adjuvant arthritis-inducing components. Further, this publication describes certain lines and clones of T lymphocytes selected for their reactivity to mycobacteria which can be used for producing arthritis upon inoculation into irradiated rats. One T cell line, designated A2, was found to induce arthritis upon intravenous (IV) injection into irradiated rats. The A2 cells were effective in vaccinating unirradiated rats against subsequent induction of arthritis by active immunization with mycobacteria. Cell line A2 was cloned, yielding two distinct clones, designated A2b and A2c. A2b cells induce arthritis but do not vaccinate against arthritis. Clone A2c cells do not cause arthritis, but rather vaccinate against it, and additionally can be used to treat existing adjuvant arthritis. Moreover, clones A2b and A2c are used to identify antigens associated with arthritogenicity or with suppression of arthritogenicity. Both clones respond in vitro to whole mycobacteria as well as to cartilage proteoglycan protein.

Van Eden et al. (European Patent Publication EP 262710 (4/6/88)), disclosed that a protein having an apparent molecular weight of about 64 kDa (the preparation of which is described in Thole, J.E.R. et al., Infec. Immun. 50:800-806 (1985)) is useful as a vaccine for inducing resistance against autoimmune arthritis and similar autoimmune diseases. This

publication also discloses that this protein, also known as antigen A, cross-reacted serologically with antigens present in other bacterial species, such as Mycobacteria, Escherichia, Treponema, Shigella, Salmonella, Yersinia, Nocardia, Campylobacter, and Klebsiella. Antigen A itself was not said to be arthritogenic, but could protect rats against arthritis induced by M. tuberculosis.

However, none of the references described above specifically discloses a method for inhibiting autoimmune diseases or other forms of undesired immunological responses, such as transplant rejection and graft-versus-host disease, by interfering with T cell-mediated immune reactions or antigen presentation using a peptide of defined structure shorter than a native protein.

Van Eden, W. et al., European Patent Publication EP 322990 (July 5, 1989), disclosed polypeptides having the amino acid sequence of residues 172-192 of antigen A, wherein residues 172-179 and/or 189-192 are entirely or partially absent. These polypeptides could be used as immunogens in pharmaceutical compositions, particularly as vaccines, for the alleviation and the treatment of autoimmune diseases, as well as in diagnostic compositions for the diagnosis of these diseases. This reference provided no guidance as to which residues could be substituted, and what amino acids could serve as appropriate substitutes, in order to obtain a useful polypeptide for preventing or treating autoimmune disease.

SUMMARY OF THE INVENTION

It is an object to the present invention to overcome the aforementioned deficiencies in the work described above.

It is another object of the present invention to provide peptides with improved capacity to inhibit the proliferative responses of lymphocytes.

It is a further object of the present invention to provide peptides which protect a subject from immune-related diseases, in particular, the T-cell mediated immune responses associated with the pathology in such autoimmune diseases as rheumatoid arthritis and reactive arthritis.

It is still another object of the present invention to provide peptides capable of acting as immunologically nonspecific agents to prevent rejection of transplanted tissues and organs.

According to the present invention, a peptide of at least seven amino acid residues capable of inhibiting the proliferative response of a T lymphocyte to a specific antigen, comprises an amino acid sequence which corresponds to positions 180-186 of the Mycobacterium tuberculosis protein hsp 65 having the formula TFGLQLE but differing therefrom by one to three amino acid substitutions in the positions 181-183, competes with hsp 65 for recognition by antigen reactive lymphocyte clones, such as clones A2b and A2c in experimental arthritis.

These substitutions can be of any form wherein the modified hsp 65 180-186 sequence is recognized by antigen-reactive T lymphocytes, such as T lymphocytes reactive to M. tuberculosis or any epitope thereof, preferably to hsp 65, T lymphocytes reactive to myelin basic protein (MBP), and the like, and the proliferative response of the T cells is inhibited.

Thus, the present invention provides a peptide of at least seven amino acid residues capable of inhibiting the proliferative response of a T lymphocyte to a specific antigen, said peptide comprising an amino acid sequence which corresponds to positions 180-186 of the Mycobacterium tuberculosis protein hsp 65 having the formula:

180						186
T	F	G	L	Q	L	E

but differing therefrom one to three amino acid substitutions in the positions 181-183.

Preferably, the peptide of the present invention has up to 20 amino acid residues, most preferably between 7 and 9 amino acid residues.

Preferred peptide of the present invention has the formula TFGAQLE, TFGAQLELT, TAGLQLE or TAGLQLELT.

Preferably, the above peptides are capable of inhibiting the proliferative response to hsp 65 of a T

lymphocyte reactive thereto, a proliferative response to myelin basic protein of a T lymphocyte reactive thereto, or a proliferative response to Mycobacterium tuberculosis of a T lymphocyte reactive thereto.

The present invention is further directed to a method for treating a subject having an autoimmune disease comprising administering to the subject an effective amount of a peptide of the present invention. In a preferred embodiment, the autoimmune disease is arthritis.

The present invention also provides a method for suppressing or preventing the rejection of a transplanted organ or tissue in a subject comprising administering to the subject an effective amount of a peptide according to the present invention, prior to and/or immediately after receipt of the transplanted organ or tissue.

The present invention is further directed to a T lymphocyte line or clone comprising T lymphocytes specifically reactive to any of the peptides of the present invention, and to the use of such cells in the prevention or suppression of an immune-mediated disease, preferably arthritis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram showing two amino acid substitution networks for the nonapeptide comprising positions 180-188 of the mycobacterial 65 kD protein, hsp 65. Figure 1a presents the "single substitution network," wherein every residue of the 180-188 sequence was replaced by each of 19 different amino acids. Figure 1b presents the "combination network," wherein substitutions at positions 180, 181, and 186 were combined.

Figure 2 is a graph showing proliferative responses of the T cell clone, A2c, to the stimulatory hsp 65 180-188 peptide, and their inhibition by alanine-substituted peptides, where the competitive peptides were added prior to stimulation.

Figure 3 is a graph showing reversal of A183 inhibition of T cell proliferation by increasing concentrations of peptide 180-188.

Figure 4 is a graph showing proliferative responses of the T cell clone, A2b, to the stimulatory hsp 65 180-188 peptide, and their inhibition by alanine-substituted peptides, where the competitive peptides were added at the time of stimulation.

Figure 5 includes graphs showing results of experiments which demonstrate the dose dependency of the inhibitory activities of A183.

Figure 6 is a graph showing that responses of an encephalitogenic T cell clone, Z1a, specific for myelin basic protein, are inhibited by the A183 peptide.

Figure 7 is a graph showing proliferative responses to various doses of A183 of lymph node cells obtained from animals either immunized or not immunized with A183.

Figure 8 is a graph showing that co-administration of A183 and an arthritogenic dose of Mycobacterium tuberculosis reduces the severity of arthritis.

Figure 9 is a graph showing the in vitro proliferative responses of clone A2b T cells to single amino acid substitution variants of peptide 180-188.

Figure 10 and Figure 11 are graphs showing proliferative responses of T cell clone A2b (Fig. 10) and T cell line Z1a (Fig. 11) to single alanine substituted variant peptides of peptide 180-188 corresponding to hsp 65 (for A2b) and of peptide 1020 corresponding to MBP (for Z1a). The peptide 180-188 series was tested at concentrations of approximately 2-3 μg/ml, and the peptide 1020 series at concentrations of approximately 50 μg/ml. Data are expressed as stimulation index (SI):

$$\frac{\text{mean cpm in the presence of peptide}}{\text{mean cpm in the absence of peptide}}$$

Figure 12 and Figure 13 are graphs showing competition for antigen presentation between non-stimulatory single alanine substituted peptide analogues and the unmodified 180-188 peptide. Non-stimulatory alanine substituted peptide analogues of peptide 180-188 (Fig. 12) and peptide 1020 (Fig. 13) were preincubated with antigen presenting cells and A2b T cells 2 h before the addition of

the stimulatory peptide 180-188. Competition was assessed by determining the reduction of proliferation in the presence of varying concentrations of the competitor peptide, stimulated by a suboptimal concentration of the stimulatory peptide 180-188 (0.5 μg/ml). Unstimulated control values were approximately 200 cpm. Results shown are the mean cpm of triplicate cultures. Error bars represent the standard error of the mean.

Figure 14 is a graph showing the reduction of arthritis severity after disease induction with M. tuberculosis (Mt) when co-injected with peptide A183. Rats were co-immunized with Mt in incomplete Freund's adjuvant together with either PBS (n=37), peptide A183, 250 μg/rat (n=18), or peptide A184, 250 μg/rat (n=19). The results shown are the mean arthritis scores of each group. Error bars represent the standard error of the mean.

Figure 15 is a graph showing the prophylactic effect of peptide A183 on development of adjuvant arthritis. Rats were immunized at day -7 with PBS/DDA (control group n=4) or with 50 μg peptide A183/DDA (A183 group n=4) and at day 0 with Mt/IFA. The results shown from a representative experiment are the mean arthritis score of each group. Error bars represent the standard error of the mean.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is based on the unexpected discovery by the inventors that peptides at least seven amino acids, corresponding to a portion of the Mycobacterium tuberculosis hsp 65 protein, which have substituted amino acids in certain positions, but not in others, are potent generalized inhibitors of T lymphocyte proliferative responses as measured in vitro, and of immune-related diseases such as autoimmune diseases in vivo.

The peptides of the present invention are those peptides which have at least seven amino acid residues comprising an amino acid sequence which corresponds to positions 180-186 of the Mycobacterium tuberculosis protein hsp 65 having the formula:

180 186
T F G L Q L E

but differing therefrom one to three amino acid substitutions in the positions 181-183. Preferably, the peptide of the present invention has up to 20 amino acids, most preferably, between 7 and 9 amino acids.

Importantly, these peptides are recognized by the immune system (either by T cells or antigen-presenting cells) and are capable of inhibiting the proliferative response of a T lymphocyte to an antigen to which the T lymphocyte is specific. Because of this recognition and inhibitory action, the peptides of the present invention can be used as competitors for recognition by any antigen-reactive T lymphocyte, or by an antigen-presenting cell. The peptides inhibit the normal stimulation of such T lymphocytes and thereby protect a subject from an immune-related disease, such as an autoimmune disease.

In particular, substitution of positions 181 and 183, most preferably position 183, of hsp 65, especially with alanine, results in a peptide having the desired properties as taught herein, and useful for protection of a subject from an immune-related disease.

Also intended within the scope of the present invention is a peptide of at least seven amino acid residues comprising an amino acid sequence which corresponds to positions 72-85 of guinea pig myelin basic protein (MBP)

72 85
Q K S Q R S Q D E N P V

and differing from the native MBP sequence by one to three amino acid substitutions, at least one of these substitutions being at position 79. Preferably, in this peptide, at least one substitution is with alanine. A preferred peptide corresponding to the MBP sequence has the formula QKSQRSQAENPV.

Identification of substituted peptides which are useful in the methods of the present invention can be easily accomplished by testing their ability to inhibit proliferative

responses in vitro of T cells, as exemplified by the arthritis-related rat T cell clones A2b and A2c.

The term "immune-related disease" as used herein refers to a disease in which the immune system is involved in the pathogenesis of the disease, or in which appropriate stimulation of the immune system can result in protection from the disease. A preferred example of an immune-related disease to which this invention is directed is an autoimmune disease. Non-limiting examples of such autoimmune diseases are rheumatoid arthritis, reactive arthritis, myasthenia gravis, multiple sclerosis, systemic lupus erythematosus, autoimmune thyroiditis (Hashimoto's thyroiditis), Graves' disease, inflammatory bowel disease, autoimmune uveoretinitis, and polymyositis.

According to the present invention, peptides are provided which can compete efficiently with Mycobacterium tuberculosis, the native Mycobacterium tuberculosis hsp 65 protein,, or its native peptide 180-188 for recognition by T lymphocytes which are associated with an immune-related disease, such as the arthritis-specific T lymphocyte clones, A2b and A2c. In another embodiment, the peptides compete with MBP for recognition by the MBP-specific and EAE-inducing T cell line, Z1a.

Preferably, the peptides of the present invention are substituted hsp 65 peptides which can compete efficiently with the native hsp 65 peptide 180-188 for recognition by A2b and A2c T cell clones, measured as inhibition of native hsp 65-induced, or native peptide 180-188-induced proliferation by the clones.

A most preferred embodiment is the 180-186 or 180-188 peptide of hsp 65 with alanine at position 183 (A183). Such a substitution creates a particularly effective competitor for binding to the T cell, presumably to the T cell receptor (TCR), the MHC molecule of the antigen-presenting cell, or both. The A183 substitution also creates a peptide particularly effective in inhibiting activation and proliferation of arthritis-specific T cells. Additionally, because of its immunogenicity (see Examples), A183 can induce

T cells specific for itself, which apparently counteract the deleterious autoimmune response in vivo. Therefore, A183 is considered to be not merely a competitor peptide but a "competitor-modulator" peptide.

By the term "arthritis-specific T cell" is intended any T lymphocyte, T lymphocyte line or clone, which is particularly associated with arthritis by virtue of the T cell's ability to induce arthritis, vaccinate against arthritis, or otherwise modulate the induction, maintenance, or suppression of arthritis in a disease-specific manner. In particular, an arthritis specific T cell is immunoreactive in vitro or in vivo with antigens such as M. tuberculosis, hsp 65, or any amino acid sequence in the hsp 65 protein which is associated with arthritis.

Examples of substitutions resulting in T cell inhibitory activity include those in which alanine has been substituted as follows:

"peptide A181" - (F-->A at position 181);
"peptide A182" - (G-->A at position 182); and
"peptide A183" - (L-->A at position 183).

Only a few peptides with substitutions at positions 180, 181, and 186 could stimulate the activity of the T cell clones. The arthritogenic A2b clone and the protective A2c clone do not show discrimination in the fine-specificity of their responses to the native and substituted peptides. Both of these T cell clones are concluded to have identical TCRs. The difference in the in vivo reactivity of the clones is thus independent of TCR specificity and is concluded to be due to differences in effector pathways induced after antigen-binding, for example, differential lymphokine production.

The fact that only a few substituted peptides retain stimulatory activity is thought to be due to the β-sheet structure of this peptide (van der Zee, R. et al., supra). This structure is thought to confer on each residue some degree of criticality in the interaction with self MHC molecules (in the case of the antigen presenting cell (APC) and with the TCR of the reactive T lymphocyte.

Table 1 shows the sequence comparison of proteins of the HSP 65 family with the mycobacterial 65 kD 180-186 amino acid sequence. In this table, the primary sequences of the different proteins are aligned with the 180-186 sequence of the mycobacterial 65 kD proteins.

TABLE 1

SEQUENCE COMPARISON OF hsp 65 PROTEINS WITH THE MYCOBACTERIAL 65 KD 180-186 AMINO ACID SEQUENCE.

Organism	Protein	Pos. 180-186	Ref
M. tuberculosis	65 kD protein	T F G L Q L E	1
M. bovis BCG	65 kD protein	T F G L Q L E	2
M. leprae	65 kD protein	T F G L Q L E	3
Escherichia coli	groEL	G L Q D E L D	4
Coxiella burnetii	62 kD protein	G L E N A L E	5
Sacch. cerevisiae	sp 60	T L E D E L E	6
Human	P1	T L N D E L E	7
Ricinus communis	Rubisco binding protein	S F E T T V D	4
Triticum aestivum	Rubisco binding protein	S F E T T V E	4

References: 1. Shinnick, T.M. J. of Bacteriol. 1987. 169:1080; 2. Thole, J.E.R. et al., Microb. Pathogen. 1988. 4:71; 3. Mehpa, V. et al., Proc. Nat'l Acad. Sci. (USA) 1986. 83:7013; 4. Hemmingson, S. et al., Nature 1988. 333:330; 5. Vodkin, H.M. et al., J. Bacteriol. 1988, 170:1227; 6. Reading, D.S. et al., Nature 1989. 337:655; Jindal, S. et al., Mol. Cell. Biol., 1989, 9:2279

Despite the overall homology of about 40-60% with the mycobacterial hsp 65, the 180-186 sequence appears to be relatively non-conserved, with only three amino acids identical (Jindal et al., supra). A synthetic peptide based on the human P1 sequence is non-stimulatory to clone A2b and A2c, as was expected.

However, in comparing the results of the single substitution network with all currently known corresponding 180-186 sequences of the 65 kD heat shock proteins of different organisms, as shown in Table 1, it was noted that the better stimulatory substitutions, position 180 T-->S, 181 F-->L, and 186 E-->D, were exclusively those amino acids that

were found to be present at the same positions in the 65 kD heat shock protein of both prokaryotic and eukaryotic organisms (note boxed residues). In 7 of 9 organisms, either threonine or serine was present at position 180. In all 9 organisms, only phenylalanine or leucine was present at 181, and only glutamic acid or aspartic acid was present at position 186.

In addition to replacing the native sequence of the 180-188 peptide with alanine, other amino acid residues can also be used. For a detailed description of protein chemistry and structure, see Schulz, G.E. et al., Principles of Protein Structure, Springer-Verlag, New York, 1978, and Creighton, T.E., Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, 1983, which are hereby incorporated by reference. The types of substitutions which may be made in the peptide of the present invention are those which will not destroy the β-sheet structure of the peptide and not interfere with the ability of the peptide to interact with MHC molecules of the host cells. The substitutions are preferably with amino acids similar to ala, that is small aliphatic, nonpolar or slightly polar residues: ser, thr, or gly. However, other amino acid substitutions are not excluded.

Whenever the term "peptide" is used in the present specification or claims, the term is intended to include a "chemical derivative" thereof which retains at least a portion of the function of the peptide which permits its utility in preventing or inhibiting T cell proliferative responses and autoimmune disease.

A "chemical derivative" of the peptide of the present invention contains additional chemical moieties not normally a part of the peptide. Covalent modifications of the peptide are included within the scope of this invention. Such modifications may be introduced into the molecule by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. Many such chemical derivatives and methods for making them are well-known in the

art. See, for non-limiting examples, U.S. Patents 3,969,287, 3,691,016, 4,195,128, 4,247,642, 4,229,537 and 4,330,440; T.E. Creighton, Proteins: Structure and Molecule Properties, W.H. Freeman & Co., San Francisco, (1983)).

Also included in the scope of the invention are salts of the peptides of the invention. As used herein, the term "salts" refers to both salts of carboxyl groups and to acid addition salts of amino groups of the protein or peptide molecule. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases such as those formed for example, with amines, such as triethanolamine, arginine, or lysine, piperidine, procaine, and the like. Acid addition salts include, for example, salts with mineral acids such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids such as, for example, acetic acid or oxalic acid.

Also included within the scope of the present invention are the peptides described herein attached to various carriers or immobilized matrices, as is well-known in the art.

It is also understood that enzymatic degradation of the peptides of the present invention in vivo may cause the peptides to be relatively short-lived. One method of preventing such degradation would be by making synthetic peptides containing a D-amino acid.

One possible modification would be to extend the peptide by moieties intended to affect solubility, e.g, by the addition of a hydrophilic residue, such as serine, or a charged residue, such as glutamic acid. Furthermore, the peptide could be extended for the purpose of stabilization and preservation of a desired conformation, such as by adding cysteine residues for the formation of disulfide bridges.

Another reason to modify the peptides would be to permit their detection after administration. This can be done by radioiodination with a radioactive iodine isotope,

directly, or by adding tyrosine for subsequent radioiodination, as discussed above.

The first requirement for an inhibitory peptide in accordance with the present invention is that it be a ligand for the TCR and/or an MHC molecule such that the peptide is recognized by the T cell or antigen-presenting cell of interest, such as the A2b and A2c clones, with sufficient affinity to compete successfully for binding with a native or other stimulatory protein or peptide (such as native hsp 65 or a stimulatory peptide derived therefrom).

In addition to the peptides described herein, the present invention provides T lymphocytes specific for these competitor-modulator peptides. An example of such an A183-specific T cell line similar in the rat is the ATL line described in Example XIV. Such cells are produced by appropriate immunization in vivo and restimulation in vitro, as discussed below. Included within the scope of the present invention are human T cells specific for the peptides of the invention, preferably derived from the individual who will be the subject of the prevention or treatment of immune-related disease, such as arthritis. Preferably such T cell lines are treated in a way which will permit their preventative or therapeutic efficacy, while inactivating them for any potential pathogenetic activity. Methods for inactivating cells or otherwise rendering them fit for vaccination against autoimmunity are well-known in the art, many of them having been developed by some of the present inventors. Such methods include treatment with hydrostatic pressure, with chemical cross-linking agents such as glutaraldehyde, with a cytoskeletal disrupting agent such as cytochalasin B, or with low doses of cells (U.S. Patents 4,634,590 and 4,716,038; Cohen, I.R. et al., European Patent Publication EP 261648; Cohen, I.R., European Patent Publication EP 291046; Cohen, I.R., Immunol. Rev. 94:5 (1986); Lider, O. et al., Proc. Nat. Acad. Sci. USA 84:4577 (1987); Lider, O. et al., Science 239:181 (1988), all of which references are hereby incorporated by reference).

Because of the close relationship at the level of antigen specificity (and presumably at the T cell receptor level) between the T cells specific for the competitor-modulator peptides of the present invention, as exemplified by ATL cells, and disease-inducing T cells (such as the A2b T cells), the T cells of the present invention are expected to induce regulatory anti-idiotypic or anti-clonotypic responses directed against a shared T cell receptor idiotope of the disease-inducing T cells. By inhibiting the generation or action of disease-inducing (e.g., arthritogenic) T cells, such a regulatory response could prevent the development of the disease.

Peptides according to the present invention are administered to patients having, or known to be susceptible to, an immune-related disease in amounts sufficient to protect the patient from the disease by preventing the patient's immune system from activation leading to induction, maintenance or exacerbation of the disease state.

Among the immune-related diseases contemplated within the scope of the present invention are also diseases involving graft rejection or graft-versus-host disease. Thus, for example, the peptides of the present invention, in particular the A183 peptide, are administered to organ transplant recipients prior to receipt of the transplanted organ, immediately after receipt of the transplanted organ, or both, in order to suppress or prevent rejection of the transplanted organ. The term "immediately after" as used herein is intended to include administration beginning in the first few hours post-transplant. However the term is not intended to limit the duration of treatment post-transplant, which can be readily determined by one of skill in the art can without undue experimentation.

In graft-versus-host disease, which may occur following bone marrow transplantation or in conjunction with pregnancy (wherein maternal alloreactive T cells may enter the fetus), T lymphocytes which recognize foreign histocompatibility antigens are transferred from donor to recipient and react against the recipient leading to a variety

of inflammatory consequences which may lead to significant morbidity and even mortality (See, for example, Roitt, I. et al., Immunology, The C.V. Mosby Company, Gower Medical Publishing, London, 1985). Thus, in one embodiment, the peptides of the present invention are administered to an individual susceptible to a graft-versus-host reaction, such as a bone marrow transplant recipient or a susceptible pregnant woman, to prevent the recognition of foreign cells in the "host" by the graft, thereby treating a graft-versus-host reaction.

The dose ranges for the administration of the compositions of the present invention are those large enough to produce the desired effect, whereby, for example, an immune response to a stimulatory peptide, as measured by T cell proliferation in vitro or a delayed hypersensitivity response in vivo, is substantially prevented or inhibited, and further, where the immune-related disease is significantly treated. The doses should not be so large as to cause adverse side effects, such as unwanted cross reactions, generalized immunosuppression, anaphylactic reactions and the like.

Effective doses of the peptides of this invention for use in treating an immune-related disease are in the range of about 1 ng to 100 mg/kg body weight. A preferred dose range is between about 10 ng and 10 mg/kg. A more preferred dose range is between about 100 ng and 1 mg/kg. The effective T lymphocyte dose is a function of the individual T cell line, the subject and his clinical status, and can vary from about 10^6 to about 10^9 cells/kg body weight. The dosage administered will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The route of administration may include intravenous, subcutaneous, intraarticular, intramuscular, by inhalation, intraperitoneal, intranasal, intrathecal, intradermal, trans-dermal or other known routes, including the enteral route.

The therapeutic use of the present invention in the treatment of disease or disorders will be best accomplished by those of skill, employing accepted principles of treatment.

Such principles are known in the art, and are set forth, for example, in Braunwald, E. et al., eds., Harrison's Principles of Internal Medicine, 11th Ed., McGraw-Hill, New York, N.Y. (1987).

In addition to peptides and T cells of the invention which themselves are pharmacologically active, pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The pharmaceutical compositions of the invention may be administered to any animal which may experience the beneficial effects of the compositions of the invention. Foremost among such animals are humans, although the invention is not intended to be so limited.

The pharmaceutical compositions of the present invention may be administered by any means that achieve their intended purpose, for example, by the routes described above. Alternatively, or concurrently, administration may be by the oral route. The peptides, T cells and pharmaceutical compositions can be administered parenterally by bolus injection or by gradual perfusion over time.

To enhance delivery or bioactivity, the peptides can be incorporated into liposomes using methods and compounds known in the art.

Preparations which can be administered orally in the form of tablets and capsules, preparations which can be administered rectally, such as suppositories, and preparations in the form of solutions for injection or oral introduction, contain from about 0.001 to about 99 percent, preferably from about 0.01 to about 95 percent of active compound(s), together with the excipient.

Suitable excipients are, in particular, fillers such as saccharides, for example lactose or sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch paste, using, for example, maize starch, wheat starch, rice starch, potato

starch, gelatin, tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone.

Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, or liquid paraffin. In addition, stabilizers may be added.

Possible pharmaceutical preparations which can be used rectally include, for example, suppositories, which consist of a combination of one or more of the active compounds with a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the active compounds with a base. Possible base materials include, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

Suitable formulations for parenteral administration include aqueous solutions of the peptides in water-soluble form, for example, water-soluble salts. In addition, suspensions of the peptides as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

The peptides are formulated using conventional pharmaceutically acceptable parenteral vehicles for

administration by injection. These vehicles are nontoxic and therapeutic, and a number of formulations are set forth in Remington's Pharmaceutical Sciences, (supra). Nonlimiting examples of excipients are water, saline, Ringer's solution, dextrose solution and Hank's balanced salt solution. Formulations according to the invention may also contain minor amounts of additives such as substances that maintain isotonicity, physiological pH, and stability.

The peptides of the invention are preferably formulated in purified form substantially free of aggregates and other protein materials, preferably at concentrations of about 1.0 ng/ml to 100 mg/ml.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE I

T Cell Clones

The isolation, maintenance, and properties of the A2b and A2c helper T cell clones have been described previously (Holoshitz et al., supra). Briefly, a T cell line, A2, reactive to M. tuberculosis was first isolated from draining lymph nodes of Lewis rats immunized with M. tuberculosis in incomplete Freund's adjuvant (IFA). Subcloning of the A2 line revealed the presence of an arthritogenic T cell clone, A2b, and a protective T cell clone, A2c. Both clones recognized the same epitope, contained in residues 180-188 of hsp 65. For long term maintenance in vitro, the T cell clones were cyclically restimulated for three days with heat killed M. tuberculosis and propagated for one week in Iscove's modification of Dulbecco's Modified Eagle's Medium (Gibco), supplemented with 10% fetal calf serum (FCS), 10% EL-4 supernatant (containing various T cell growth factors), 2 mM glutamine, antibiotics, and 1% nonessential amino acids.

The isolation, maintenance and properties of the encephalitogenic Z1a helper T cell line have been described previously. Briefly, T cell line Z1a reactive to the 72-85 amino acid sequence of guinea pig myelin basic protein (MBP) was isolated from the draining lymph nodes of a Lewis rat 9 days after immunization into each foot pad with guinea pig MBP in CFA.

The helper T cell line ATL was isolated from the popliteal lymph nodes of a Lewis rat 10 days after hind footpad immunization with 100 μg/ml peptide A183 in CFA (containing 4 mg/ml of M. tuberculosis (H37Ra).

T cell lines were cyclically restimulated in vitro for 3 or 4 days with irradiated (3000 rads) thymocytes as APCs and 10 μg/ml heat-killed Mt in case of clone A2b, 10 μg/ml MBP in case of T cell line Z1a or 10 μg/ml peptide A183 in case of T cell line ATL and propagated for 6 or 7 days in Iscove's Modified Dulbecco's Medium (Gibco), supplemented with 10% FCS, 10% EL-4 supernatants (as a source of IL-2), 2 mM glutamine, 2-ME, antibiotics and 1% non-essential amino acids.

EXAMPLE II

Immunological Assays

T Cell Proliferation Assay

The proliferative response of T cell clones was assessed by measuring ^{3}H-thymidine incorporation into cells over the final 18-20 hours of culture. Cells (2×10^4 cells/well) were cultured for 4 days in flat-bottom 96-well microtiter plates in the presence of irradiated (1500 rads) syngeneic thymocytes (1×10^6 cells/well) as antigen presenting cells and various amounts of antigen. Following incorporation of the isotope, cells were harvested and counted using routine procedures.

Competition Assay

To assess the capacity of a peptide to compete for recognition (and stimulation of T cells) with the native peptide 180-188, the competitor peptide was added to the culture containing the T cells and irradiated thymocytes and responder cells 1-2 hours before the addition of the

stimulatory peptide 180-188. Competitive activity was evaluated by determining either the concentration of the stimulatory peptide required to achieve positive proliferation in the presence of a fixed concentration of the competitor peptide, or alternatively, as the concentration of competitor peptide required to achieve a statistically significant reduction in proliferation stimulated by a suboptimal concentration of the stimulatory peptide (typically 0.5 μg/ml).

EXAMPLE III

Preparation of Antigens and Peptides

Large quantities of synthetic peptide 180-188, TFGLQLELT, peptide A183, TFGAOLELT, peptide A184, TFGLALELT, peptide 1020, QKSORSQDENPV, peptide 1028, QKSQRSQAENPV, and peptide 1029, QKSQRSQDANPV were synthesized by the solid phase technique (Van Eden, W. et al., Nature 1988, 331:171; Steward, J.M. et al., Solid phase peptide synthesis (Pearce Chemical Company, Rockford, Illinois, 1984)). The single alanine substituted peptide analogs derived from non peptide 180-188, the 180-188 amino acid sequence of the mycobacterial 65 kD protein, were synthesized with the PEPSCAN method (Geysen, H.M. et al., Proc. Natl. Acad. Sci. USA, 1984, 81:3998; Proc. Natl. Acad. Sci. USA 1985, 82:178)) and detached from their solid supports and used in T cell proliferation assays as described previously (van der Zee et al., supra). The single alanine substituted peptide analogs derived from peptide 1020, the 72-85 amino acid sequence of guinea pig MBP, were prepared by automated simultaneous multiple peptide synthesis (SMPS). The SMPS set-up was developed using a standard autosampler (Gilson model 221). Shortly, for the concurrent synthesis of 30 peptides standard Fmoc-chemistry with Pfp-activated amino acids (Dhbt for serine and threonine) in a sixfold molar excess and Hobt as catalyst were employed. Peptides were obtained as C-terminal amides from 7.5 mg resin/peptide (0.21 meq/g, PAL TM resin, Milligen). The activities of in vitro defined non-inhibitory and inhibitory peptides synthesized by the PEPSCAN

or the SMPS method were confirmed with the alanine substituted peptides, A184, 1029 and A183, 1028 prepared by conventional solid phase synthesis. Heat-killed Mycobacterium tuberculosis (Mt) H37Ra was derived from DIFCO Laboratories, Detroit, Michigan). The 65 kD Mycobacterium bovis BCG recombinant protein was cloned and purified as described previously (Thole et al., 1987; Van Eden et al., 1988).

Two types of amino acid replacement "networks" were prepared. In the "single substitution network," every residue of the 180-188 sequence was replaced by each of 19 different amino acids (Figure 1a). In the "combination network" substitutions at positions 180, 181, and 186 were combined (Figure 1b).

To detach peptides from their solid support (Van der Zee, R. et al., supra), 70% formic acid was used to cleave an Asp-Pro bond which is part of a tripeptide Asp-Pro-Gly, extended at the C-terminus of the peptides. Formic acid was removed by evaporation and remaining dry peptide was dissolved directly without further purification. The activities of competitive peptides were confirmed with alanine-substituted peptides, A183 and A184, synthesized by the conventional solid phase technique.

EXAMPLE IV

PEPTIDE A183 INHIBITS PROLIFERATIVE RESPONSES IN VITRO OF T CELLS OF CLONES TO NATIVE PEPTIDE 180-188

Proliferative responses of the T cell clones and the capacity of synthetic peptides to compete with the native stimulatory peptide 180-188 were assessed as described above. Figure 2 shows response of A2c cells to a suboptimal concentration of the native peptide 180-188 in the absence or presence of alanine-substituted peptides. Preincubation of T cells and APC with 10 µg/ml of A183 (hsp 65 180-188 substituted by alanine at position 183) caused almost complete inhibition of the response. A181 was also inhibitory, although to a lesser extent. A peptide with an alanine substitution at position 182 showed only slight inhibition.

Peptides with substitutions at positions 180. 184, 185, or 186 failed to inhibit responses to the stimulatory peptide.

To exclude the possibility that the observed inhibition was due to toxic effects of the selected alanine-substituted peptides, inhibitory peptides were tested in the presence of increasing concentrations of stimulatory peptide 180-188. As can be seen from Figure 3, the stimulatory 180-188 peptide overcame inhibition in a dose-dependent manner. Therefore, the inhibition by the substituted peptides followed the rules of dose-dependent competition. Furthermore, responses to the T cell mitogen, concanavalin A (Con A), were not affected by the presence of alanine-substituted peptides in the concentrations which could inhibit the 180-188 response.

Figure 4 shows that, when the competitor peptides were added at the same time as the stimulatory peptide (here at 0.1 μg/ml), without preincubation, A183 competed for recognition by A2b cells, but A181 did not.

Figure 5 shows a distinct set of experiments which demonstrate the dose-dependency of the inhibitory activities of A183. In Figure 5a-5c, A2b cells were incubated with APC and a fixed concentration of A183 (10 μg/ml). After 1-2 hours of preincubation, increasing concentrations, from 0.1 - 10 μg/ml of stimulatory antigens were added (Figure 5a: peptide 180-188; Figure 5b: hsp 65; Figure 5c: M. tuberculosis). In Figure 5d-5f, the concentration of stimulatory antigens was fixed at 1 μg/ml, while the concentration of A183 was varied between 10 and 0.1 μg/ml. Competition was evaluated as described above.

Figure 5a shows that at a fixed concentration of A183 (10 μg/ml), positive responses to native peptide 180-188 were observed only at high concentrations of the stimulatory peptide (10 μg/ml). At equimolar concentrations, inhibitory peptide A183 was a very efficient competitor.

Figure 5b and Figure 5c show that the T cell proliferative response to the entire hsp 65 protein (depicted as "65 kD") and even to killed whole M. tuberculosis (depicted as "Mt") were inhibited efficiently by A183.

Figure 5d, 5e and 5f show the concentration-dependence of the inhibitory effect of A183 on responses to native peptide 180-188, hsp 65 and whole M. tuberculosis.

EXAMPLE V

A183 INHIBITS PROLIFERATION OF MBP-SPECIFIC ENCEPHALITOGENIC T CELLS (Z1a)

In this example, the T cell specificity and the disease specificity of the inhibitory effect mediated by A183 was tested.

In the experimental rat model of allergic encephalomyelitis (EAE), disease can be induced by immunization with the basic protein of myelin (MBP) or by inoculation of MBP-reactive T cell lines, such as Z1a (Ben-Nun, A. et al., J. Immunol. 129:303 (1982)).

Cells of the T cell line Z1a was incubated with irradiated thymocytes (APC) and a fixed concentration of A183 (10 μg/ml). After 1-2 hours preincubation, the stimulatory antigen, myelin basic protein (MBP), was added in concentrations increasing from 0.1 - 10 μg/ml. Competition was evaluated as described above.

Figure 6 shows that proliferative responses of Z1a to MBP are inhibited by A183. The Z1a cell line is known to recognize the amino acid sequence of residues 68-88 of MBP, which bears no sequence or structural similarity to residues 180-188 of hsp 65.

Therefore, A183 seems to inhibit responses of both the arthritis-specific T cell clones and an encephalitis-specific T cell line by competing for binding at the level MHC class II molecules (Rt-1 B locus products) involved in antigen presentation.

EXAMPLE VI

ANTIGEN-SPECIFIC PROLIFERATION OF RAT LYMPH NODE CELLS AFTER IMMUNIZATION WITH A183

Studies were performed to test whether the competitive and inhibitor peptide, A183, can be bound and

presented by the MHC molecules of antigen-presenting cells. Such binding and presentation can be inferred from a showing that immunization of rats in vivo with peptide A183 leads to the generation of lymph node cells capable of responding to A183 in vitro.

Lewis rats were immunized subcutaneously (SC) in their hind footpads with A183 (500 μg/rat) in IFA, or with IFA alone. Sixteen days later, rats were immunized intracutaneously at the base of the tail with 1 mg of heat-killed M. tuberculosis in IFA. Eleven days later, the popliteal lymph nodes (LN) were removed and the T cells were tested in a standard proliferation assay using 2×10^5 cells/well and various concentrations of A183, ranging from 1 - 50 μg/ml. ^{3}H-thymidine incorporation was measured after 4 days of culture.

Figure 7 shows proliferative responses of immune or control LN cells to various doses of A183, indicating that, under appropriate circumstances, A183 can function as an immunogenic peptide. These results also show that, even at high concentrations (50 μg/ml), A183 has no inherent toxic action on T cells.

EXAMPLE VII

A183 REDUCES THE SEVERITY OF M. TUBERCULOSIS-INDUCED ARTHRITIS

The response to hsp 65 residues 180-188 by the A2b T cell clone is dominant in adjuvant arthritis, and the response to MBP by the T cell clone Z1a is dominant in experimental allergic encephalomyelitis. Since A183 inhibited these clonal T cell reactivities in vitro, the in vivo effects of A183 administration at the time of disease induction were investigated.

Active arthritis was induced in Lewis rats by intracutaneous inoculation of 200 μg M. tuberculosis in IFA. In animals receiving A183, 100 μg of the inhibitory peptide was added to the inoculum. The system described by Trentham et al. (J. Exp. Med. 146:857-868 (1977)), was used to assess the severity of the arthritis. Each paw was graded from 0 to 4 based on erythema, swelling, and deformity of the joint. The

highest score achievable was 16. On different days after immunization the arthritis score was determined. The results, shown in Figure 8, represent the mean arthritis score of five (control group) or seven (A183 group) animals. The standard deviation is indicated by bars.

Figure 8 shows that the concomitant administration of A183 with M. tuberculosis caused a significant reduction in the severity of arthritis ($p<0.02$). The disease developing in A183-treated rats was very mild and did not cause irreversible joint damage (average maximum disease score of 4). Animals which did not receive A183 developed much more severe disease with irreversibly ankylosed joints.

EXAMPLE VIII

A183 REDUCES THE SEVERITY OF MBP-INDUCED EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

In a study performed essentially in the same way as the arthritis study in Example VII, above, it was found that A183 given at the time of immunization with MBP resulted in reduction of the severity of EAE.

EAE was induced in Lewis rats by injecting 0.1 ml of a 1:1 emulsion of MBP in saline and complete Freund's adjuvant (containing 4 mg/ml M. tuberculosis) in both hind footpads. Clinical signs of disease were monitored daily, and rated on a scale of 0 to 4: 0, no signs; 0.5, lethargy and weight loss; 1, limp tail; 2, hind leg weakness; 3, hemiparalysis; 4, paralysis of front and hind limbs, moribund condition. Animals received either 50 μg of MBP (in adjuvant) alone or 50 μg MBP plus 500 μg of A183. The results are shown in Table 2.

In the A183-treated group, only 1 of 4 rats developed any paralysis, while in the MBP controls 3 of 4 rats developed disease.

TABLE 2

A183 REDUCES THE SEVERITY OF MBP-INDUCED EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

	MBP				MBP + A183			
Inoculated Rat #:	1	2	3	4	5	6	7	8
EAE Score:	1	1	2	0.5	0.5	0.5	0.5	1
Total Weight Loss: (mg)	40	58	55	45	36	31	27	46
Days With Weight Loss:		10/16				13/17		

In a follow-up experiment, a different route of immunization for EAE induction was used, wherein 0.1 ml was injected intradermally in the base of the tail. Again, a significant delay in development of disease was observed. Furthermore, based on body weights, the A183 inoculated animals were healthier than the controls.

The *in vitro* proliferative responses of T cells to MBP antigens were measured 34 days after induction of EAE. Table 3 shows that in rats which had received A183 co-administered with MBP, there was a reduced level of T cell priming to MBP. Such reduced responsiveness to MBP was not the result of generalized immunosuppression, since T cells from rats which had received A183 responded to A183 antigen *in vitro*, and had normal polyclonal T cell responses to Con A.

TABLE 3

IN VITRO PROLIFERATIVE RESPONSES OF T CELLS FROM RATS GIVEN A183 IN EAE

Incorporation of ^{3}H-thymidine (cpm x 10^{-3})

In Vitro Stimulus	Conc. (μg)	MBP Inoculated 1	2	3	4	MBP + A183 Inoculated 5	6	7	8
MBP	0.1	1.4	1	1.3	1.2	1	1	1	1
MBP	1	1.9	1	2	1.6	1	1	1.6	1.5
MBP	10	4.5	4	7.4	3.6	2.6	2	3.3	2.4
MBP	20	6.3	5.3	7.8	4.4	2.7	1.7	3.3	3.2
A183	1	1.2	1	1	1	1.4	1	1.4	1.5
A183	10	1	1	1	1	5.0	4.2	5.2	4.8
A183	50	1.6	1	1	1.2	7.2	8.8	8.4	9.5
Con A	2.5	70	446	33	14	35	74	29	121

EXAMPLE IX

A183 INHIBITS PROLIFERATION OF HUMAN T CELLS TO M. TUBERCULOSIS ANTIGENS

To see whether the inhibitory activity of A183 was exerted not only on T cells of differing antigen specificity but also on T cells restricted to differing MHC molecules, the action of A183 on proliferative responses of human T cells was assessed.

Table 4 shows M. tuberculosis responses of peripheral blood lymphocytes obtained from patients with chronic rheumatoid arthritis. Without exception, positive responses to the crude M. tuberculosis antigen were inhibited, up to 76%, by A183. Cells from patients showing very low responsiveness to the antigen (patients DS and VOS) were not inhibited by A183. Such low responses are thought to reflect non-specific reactions to mitogenic substances present in the M. tuberculosis preparation rather than a low antigen-specific response; such non-specific responses are not expected to be inhibited by the peptide.

TABLE 4

INHIBITION OF ANTIGEN-INDUCED PROLIFERATION OF HUMAN LYMPHOCYTES TO M. TUBERCULOSIS (MT) BY A183

	Incorporation of ^{3}H-thymidine (Net cpm)		
Patient	MT	MT + A183	% INHIBITION
BB	5874	1612	72
TER	3574	2440	32
DU	1794	421	76
KOR	9658	5816	32
LON	1723	1153	33
ELA	1651	759	54
RIJ	803	668	17
DS	472	469	1
VOS	845	852	0

M. tuberculosis is a crude antigenic preparation containing a large number of potentially antigenic epitopes. Genetically different individuals express different MHC products. It is assumed that different MHC products present different epitopes of the antigen selected from the large number available in the crude preparation. Despite this compounded variability, the A183 peptide appears to be capable of interacting with different MHC products, both rat and human. A183 not only inhibits antigen specific T cell recognition in both species, but in addition, it effectively interferes with the in vivo generation of autoimmune effector T cells, as shown in adjuvant arthritis and allergic encephalomyelitis experiments, above.

EXAMPLE X

INVESTIGATION OF STIMULATORY AMINO ACID SUBSTITUTED PEPTIDES

A. Single Amino Acid Substitutions

To investigate which residues in the nonapeptide TFGLQLELT were critical for stimulation of the A2b and A2c T cell clones and which substitutions could be permitted for recognition, a single substitution network of peptide 180-188 was prepared. In this network every residue of the

nonapeptide was replaced by all 19 other possible amino acids (See Figure 1).

Proliferative responses of both A2b and A2c cells to stimulation by all the single substituted 180-188 variant peptides were determined. Figure 9 shows the results for the A2b T cell clone. The results for clone A2c were found to be comparable. Table 5 presents a limited array of results for substitutions at positions 180, 181 and 186.

TABLE 5

T CELL PROLIFERATIVE RESPONSE TO PEPTIDE 180-186 VARIANTS

Peptide 180 186	Proliferative Response of Clone A2b (SI)
T F G L Q L E	174
S - - - - - -	140
- L - - - - -	85
- - - - - - D	16
S L - - - - -	1
S - - - - - D	1.5
- L - - - - D	1
S L - - - - D	0.8

A) Shown are all possible combinations of 3 stimulatory single substitution peptides. Unchanged residues are indicated with a dash. The peptide concentrations used were approximately 4 μg/ml. The data are expressed as stimulation indices (SI), which is calculated as:
mean cpm (plus antigen) / mean cpm (minus antigen
Similar results were obtained with clone A2c.

All peptides with substitutions at positions 187 and 188 stimulated both T cell clones equally well, and no difference was observed from the original peptide.

Remarkably few replacements were permitted within the critical 180-186 sequence. At positions 182, 183, 184, and 185, none of the substituted peptides was stimulatory, while of the peptides substituted at positions 180, 181, and 186, only a few stimulated the T cell clones.

Substitution peptides that induced high proliferative T cell responses (SI > 10) were: position 180 T-

->S; position 181 F-->L; and position 186 E-->D or E-->Q. Peptides having the following substitutions were also stimulatory for both T cell clones: position 180 T-->D or T-->K; position 182 F-->I; position 186 E-->H. However, the degree of proliferative responses found in the presence of the latter substitutions peptides was clearly inferior to those obtained with native peptide 180-188.

B. Substitution Peptides with 2 or 3 Replacements

Substitution peptides with two or three replacements were found to be non-stimulatory. Based upon the results obtained with the single substitution network, the better stimulatory substitutions, position 180 T-->S, position 181 F-->L, and position 186 E-->D were selected for synthesis of combined substitution peptides. Proliferative responses of the A2b and A2c T cell clones to the combined-substitution peptides were determined.

As can be seen from the results for clone A2b, shown in the lower half of Table 5, none of the peptides with two or three combined substitutions at positions 180, 181, and 186 was stimulatory.

EXAMPLE XI

IDENTIFICATION OF NON-STIMULATORY ALANINE-SUBSTITUTED PEPTIDES AND THEIR CAPACITY FOR COMPETITIVE INHIBITION

For the design of competitor peptides based on the two disease-associated T cell epitopes, the 180-188 amino acid sequence of the mycobacterial 65 kDa protein and the 72-85 amino acid sequence of guinea pig MBP (designated peptide 1020), tests were conducted to determine which residues within these epitopes were essential for stimulation of T cell clone A2b and T cell line Z1a, measuring proliferative responses of these T cells to substituted peptides. As can be seen in Figure 10, T cell clone A2b responded to the native peptide 180-188 and to the alanine-substituted peptides A187 and A188. Figure 11 shows that T cell line Z1a responded not only to the native MBP peptide 1020 but also to the alanine-substituted peptides 1021, 1024, 1026, 1030, and 1032, and a very modest

response to peptide 1031 was noted. Peptide 1026 induced a larger proliferative response than did peptide 1020.

Because both A2b and Z1a cells were restricted by the MHC class II RT1 B^{l} locus (I-A) (Boots, A.M.H. et al. J. Immunol. Methods, 1991, in press), clone A2b was used to test the inhibitory capacity of non-stimulatory alanine-substituted peptides of the 180-188 series and the peptide 1020 series. As can be seen from Figure 12, A181 and A183 showed a concentration-dependent inhibition of the response to peptide 180-188 (0.5 μg/ml). Peptide A182 showed only slight inhibition, while A180, A184, A185 and A186 showed no competitive inhibition.

As is shown in Figure 13, of the substituted peptide 1020 series, only peptide 1028 caused a strong concentration-dependent inhibition of proliferation of clone A2b in response to peptide 180-188, comparable in potency to the inhibition caused by A183.

When a fixed dose of inhibitory peptide A183 or 1028 was tested in the presence of increasing concentrations of stimulatory peptide 180-188, the inhibition could be overcome in a dose-dependent manner, arguing against any non-specific toxic effects of the inhibitory peptides on the T cells. Furthermore, both A183 and 1028 inhibited the proliferative response of Z1a cells to either MBP or peptide 1020, whereas peptides A184 and 1029 did not inhibit.

Based on the above results, peptide A183 and 1028 were selected for testing in the adjuvant arthritis and EAE disease models. In these studies, peptide A184 and 1029 were used as controls.

EXAMPLE XII

PEPTIDES A183 AND 1028 COUNTERACT DEVELOPMENT OF EAE

EAE was induced with the encephalitogenic MBP peptide 72-85 (i.e., peptide 1020) at 50 μg/rat, emulsified in CFA, basically as described in Example VIII. Co-immunization with peptide 1028 (500 μg/rat) resulted in a complete inhibition of EAE, while co-immunization of peptide A183 (500

μg/rat) resulted in almost complete inhibition (Table 6). Only 2 of 8 animals co-immunized with A183 showed some clinical signs of EAE. However, the maximal disease scores of these 2 animals was 0.5 (weight loss without any signs of paralysis). In contrast, all animals co-immunized with control peptide 1029 developed severe EAE, comparable with the control disease group (Table 6).

Table 6

Co-immunization with 1028 or A183 Inhibits Peptide-induced EAE

Immunization[a]	Incidence[b]	Day of onset[c]	Duration[d]	Maximal Severity[e]
1020 (50ug) + PBS	8/8	12.9±1.4	5.8±1.7	2.0±1.1
1020 (50ug) + 1028 (500ug)	0/8	-	-	-
1020 (50ug) + A183 (500ug)	2/8	13.0±0	1 ± 0	0.1±0.2
1020 (50ug) + 1029 (500ug)	8/8	11.8±1.0	5.5±0.9	2.6±0.8

[a] EAE was induced by subcutaneous injection of 1020 (50μg) + CFA (Mt 400 μg) emulsified with PBS, 1028 (500μg), A183 (500μg) or 1029 (500 μg). Rats were observed daily and graded on a four-point scale.
[b] Number with disease/number tested.
[c] Average day of disease onset of those animals that developed disease (±SD).
[d] Average duration of the disease in days of those animals that developed disease.
[e] Value represents the mean of the maximum EAE score for each experimental group.

EXAMPLE XIII

INHIBITORY ACTIVITY OF PEPTIDES A183 AND 1028 IN ARTHRITIS

Arthritis was induced as described in Example VII. As shown in Figure 14, after arthritis induction with Mt/IFA in the presence of peptide A183 (250 μg peptide/rat) a very significant reduction of the arthritis severity was observed compared with the control groups ($p>0.001$). No significant effect on disease severity was seen when the *in vitro* non-competitive control peptide A184 was used ($p.0.05$) (Figure 14). Interestingly, addition of the EAE-related peptide 1028

(250 μg peptide/rat) to the Mt/IFA inoculum did not reduce disease ($p>0.05$).

In contrast to EAE, where disease can be induced by a well-defined MBP-related peptide, the disease-inducing antigen for adjuvant arthritis is whole heat-killed Mycobacterium tuberculosis, a complex mixture of antigens. Nevertheless, results presented above show that a single well-defined "blocking" peptide can inhibit arthritis induction (Figure 14).

On day 43 after disease induction, when the clinical signs of arthritis had subsided, inguinal lymph node (LN) cells of animals immunized with Mt/IFA together with either PBS, peptide A183, or peptide A184 were obtained and tested for antigen specific proliferation. In a representative experiment, the results of which appear in Table 7, no differences were observed between groups when LN cells were incubated with Mt or its hsp 65 protein. However, LN cells from animals co-immunized with A183 did show proliferative responses to peptide A183 itself. In this group the polyclonal proliferative response to peptide 180-188 was increased relative to controls, but there was no response to peptide A184. The group co-immunized with "control" peptide A184 showed no polyclonal proliferative responses to either peptide A184, peptide A183 or peptide 180-188.

Table 7

Proliferative Responses of Inguinal Lymph Node Cells at Day 43 After Arthritis Induction[a]

Antigen	(μg/ml)	Rats immunized at day 0 with: Mt/-	Mt/A183	Mt/A184
Mt	10	36.9	36.5	23.5
65 kD	25	3.0	4.4	5.1
180-188	10	1.6	3.7	1.2
	25	1.3	3.9	1.4
A183	10	1.5	3.8	1.2
	25	0.9	4.0	1.3
A184	10	0.9	0.8	1.3
	25	0.9	1.0	1.5
Max.Arthritis Score		16	1	16

[a] Data are expressed as SI. For disease induction, rats were immunized at day 0 with Mt (250 μg) in IFA emulsified with PBS or A183 (250μg) or A184 (250μg) in PBS.

Similarly, in the EAE disease model of Example XII, LN cells from co-immunized animals showing a proliferative response to peptide A183 always had an increased response to peptide 180-188, but no response to A184.

EXAMPLE XIV

GENERATION OF A T CELL LINE (ATL) SPECIFIC FOR A183

The nature and specificity of the polyclonal T cell responses which developed following arthritis or EAE induction in the presence of A183 indicated that (a) peptide A183 was immunogenic in Lewis rats, and (b) proliferative responses induced by peptide A183 immunization included responses against the native peptide 180-188. Based on the results, however, it was not possible to determine whether the same T cells that recognized A183 responded to peptide 180-188. For

this reason, the present inventors generated a A183-specific T cell line. Rats were immunized with 100 μg A183 in CFA in the hind footpads and, 10 days later, popliteal lymph node cells were obtained and stimulated in vitro with A183 (10 μg/ml) for 4 days. The cells were then expanded for 1 week in IL2-containing medium and restimulated for 4 days by A183 (10 μg/ml) in the presence of syngeneic irradiated thymocytes as antigen presenting cells. Table 8 shows the antigen specificity of a $CD4^+$ T cell line, designated ATL, obtained after 6 restimulation cycles in vitro. These cells were dramatically stimulated by A183. Moreover, in agreement with results at the polyclonal level, these same cells responded well to peptide 180-188, but not at all to A184, the hsp 65 or Mt. These results are in sharp contrast with all previously generated T cell lines specific for peptide 180-188 specific T cell lines, which had been obtained by selection with either whole Mt (clones A2b and A2c) or hsp 65 (clone B2) (Van Eden, W., Immunol. Rev. 121:5-28 (1991)), which do not respond to peptide A183. T cells of the ATL line recognize both peptide 180-188 and A183, but not hsp 65 or Mt.

Table 8

Antigen Specificity of T Cell Line ATL

Antigen	(μg/ml)	Proliferative response (cpm ± SD)
-	-	390 ± 10
ConA	2.5	87995 ± 1726
Mt	1	178 ± 35
	10	220 ± 40
65kD	25	512 ± 34
	50	403 ± 77
180-188	1	82720 ± 3520
	10	123194 ± 1974
A183	1	86256 ± 1552
	10	139568 ± 2318
A184	1	508 ± 102
	10	362 ± 37

Analysis of the fine specificity of ATL using sequentially overlapping peptides indicated a one residue difference in the length of the optimally recognized peptide compared with the minimal length recognized by the peptide 180-188-specific A2b clone (van der Zee et al., supra). It is concluded that the peptide structure recognized by ATL is not present after natural processing of the mycobacterial 65 kDa protein hsp 65.

In summary, co-immunization of peptide A183 together with Mt resulted in both disease reduction and triggering of T cell responses against peptide A183 and 180-188. Generation of an A183 specific T cell line (ATL) revealed that T cells recognizing peptide A183 also responded to peptide 180-188 but not to Mt or the mycobacterial 65kDa protein. This indicated that after processing of the mycobacterial 65 kDa protein, a fragment was generated that could be recognized by clone A2b but not by the peptide A183-specific T cell line, ATL. Thus, co-immunization of peptide A183 primes a population of T cells that would remain

"cryptic" upon priming with Mt or the mycobacterial 65kDa protein.

EXAMPLE XV

PROPHYLACTIC IMMUNIZATION WITH PEPTIDE A183 INHIBITS ADJUVANT ARTHRITIS BUT NOT EAE

Based on the efficient inhibition of arthritis induction by co-immunization with A183 and the fact that A183 was immunogenic and elicited a T cell responses against peptide 180-188, experiments were designed to test whether prophylactic immunization with A183 7 days before disease induction could influence the subsequent development of arthritis or EAE. Results are shown in Figure 15.

Subjects immunized with 50 μg A183 in dimethyl - dioctadecylammonium bromide (DDA) (Eastman-Kodak Company, Rochester, New York, USA), an adjuvant unrelated to mycobacteria (see, for example, Snippe, H. et al., In: Immunological Adjuvants and Vaccines, G. Gregoriades et al., eds, Plenum Press, New York, 1989, pp. 47-59), 7 days before disease induction with Mt/IFA, showed significant disease reduction compared with the control group receiving PBS/DDA at day -7 ($p<0.05$). In contrast, prophylactic immunization on day -7 with peptide A183 did not interfere with the development of EAE induced with the MBP peptide 1020 (maximal EAE score of 2.2 ± 1.2).

DISCUSSION

In the present arthritis model, immunization with attenuated A2b cells is known to induce protection by triggering disease-specific regulatory T cells (Lider, O. et al. Proc. Natl. Acad. Sci. (USA) 84:4577- 4580 (1987)). In the EAE system, it was recently demonstrated that T cells recognizing different MBP epitopes can induce cross-reactive immunity against a T cell receptor idiotope that is shared by these cells (Offner, H. et al. J. Immunol. 146:4165-4172 (1991)). Therefore, because of the close relationship at the level of antigen specificity (and presumably at the T cell receptor level) between the ATL cells of the present invention and the disease-inducing A2b-like T cells, it is proposed that

the in vivo counterparts of the ATL cells induce regulatory anti-idiotypic responses directed against a shared T cell receptor idiotope. By suppressing the arthritogenic cells (or the A2b type), this regulatory response could prevent the development of arthritis.

This notion is supported by the above findings that rats prophylactically immunized with peptide A183, 7 days before disease induction had reduced disease. In the EAE model, where disease was induced with the MBP peptide 1020 in adjuvant, prior treatment with A183 had no effect, presumably because the disease is mediated by unrelated clones of T cells not sharing T cell receptor epitopes with the A183-induced ATL-like regulatory T cells.

It is likely that inhibition of the development of arthritis was not due to competitive MHC blocking by residual A183, because (a) a very low dose of A183 was effective compared with the co-immunization experiments and (b) no effect on EAE no was seen.

In conclusion, the results presented above show that peptides can be developed that efficiently inhibit in vitro T cell proliferative by T cells restricted by the same MHC molecule but with distinct antigenic specificity. However, in vivo, disease-related competitor peptides, exemplified by A183, may show not only MHC blocking (as demonstrated in the peptide-induced EAE model) but also disease-specific suppressive activity. In adjuvant arthritis, a disease induced by a highly complex antigen, whole mycobacteria, disease inhibition by co-immunization with a competitor peptide apparently involves the synergistic effect of competition for MHC binding between competitor and disease-eliciting epitopes, as well as the concomitant induction of a regulatory (protective) immune response. The various properties of such novel competitor-modulator peptides make them attractive tools for the treatment of human autoimmune diseases, where antigens are relatively complex and multiple antigen-presenting structures are present in MHC-heterozygous individuals. In that setting, an effective therapeutic or

prophylactic peptide may have to do more than simply bind in a competitive fashion to MHC molecules.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A peptide of at least seven amino acid residues capable of inhibiting the proliferative response of a T lymphocyte to a specific antigen, said peptide comprising an amino acid sequence which corresponds to positions 180-186 of the Mycobacterium tuberculosis protein hsp 65 having the formula:

180						186
T	F	G	L	Q	L	E

but differing therefrom by one to three amino acid substitutions in the positions 181-183.

2. A peptide according to claim 1, wherein said substitution is with one alanine residue.

3. A peptide according to claim 1 having the amino acid sequence TFGAQLE.

4. A peptide according to claim 1 having the amino acid sequence TFGAQLELT.

5. A peptide according to claim 1 having the amino acid sequence TAGLQLE.

6. A peptide according to claim 1 having the amino acid sequence TAGLQLELT.

7. A peptide according to claim 1 which is capable of inhibiting the proliferative response to hsp 65 of a T lymphocyte reactive thereto.

8. A peptide according to claim 1 which is capable of inhibiting the proliferative response to myelin basic protein of a T lymphocyte reactive thereto.

9. A peptide according to claim 1 which is capable of inhibiting the proliferative response to Mycobacterium tuberculosis of a T lymphocyte reactive thereto.

10. A peptide of at least seven amino acid residues capable of inhibiting the proliferative response of a T lymphocyte to a specific antigen, said peptide comprising an amino acid sequence which corresponds to a sequence included in the positions 72-83 of guinea pig myelin basic protein having the formula:

72											83
Q	K	S	Q	R	S	Q	D	E	N	P	V

- but differing therefrom by one to three amino acid substitutions.

11. A peptide according to claim 10, wherein said substitution is with one alanine residue and said peptide has the amino acid sequence QKSQRSQAENPV.

12. A method for treating a subject having an autoimmune disease comprising administering to said subject an effective amount of a peptide according to claim 1.

13. A method according to claim 12 wherein said disease is arthritis.

14. A method for suppressing or preventing the rejection of a transplanted organ or tissue in a subject comprising administering to said subject an effective amount of a peptide according to claim 1 prior to and/or immediately after receipt of the transplanted organ or tissue.

15. A T lymphocyte cell line comprising T lymphocytes specifically reactive with a peptide according to claim 1.

16. A T lymphocyte cell line comprising T lymphocytes specifically reactive with a peptide according to claim 2.

17. A T lymphocyte cell line comprising T lymphocytes specifically reactive with a peptide according to claim 3.

18. A method for treating a subject susceptible to or having or an autoimmune disease comprising administering to said subject an effective amount of T lymphocytes according to claim 15.

FIG. 1A

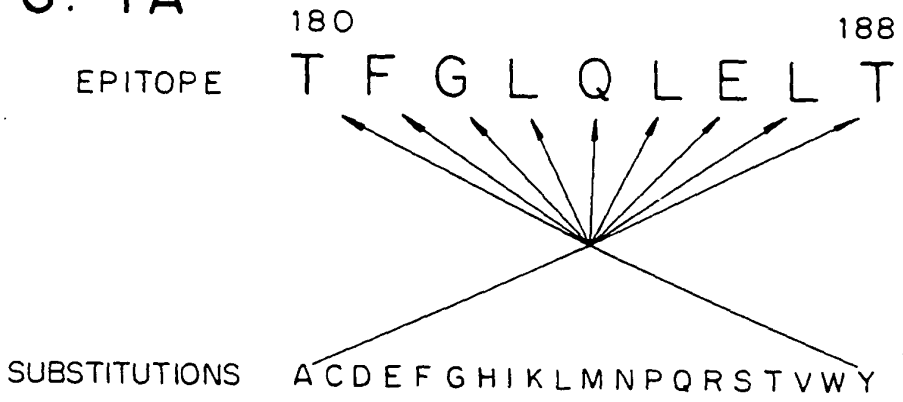


FIG. 1B

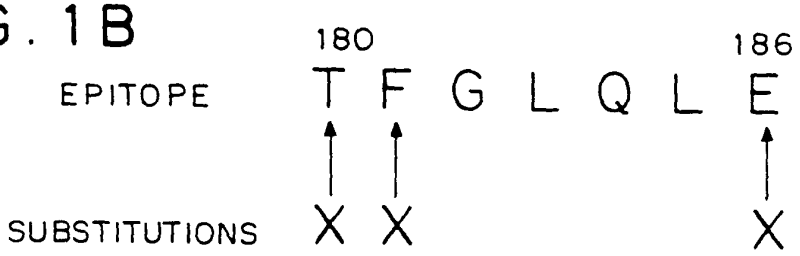


FIG. 2

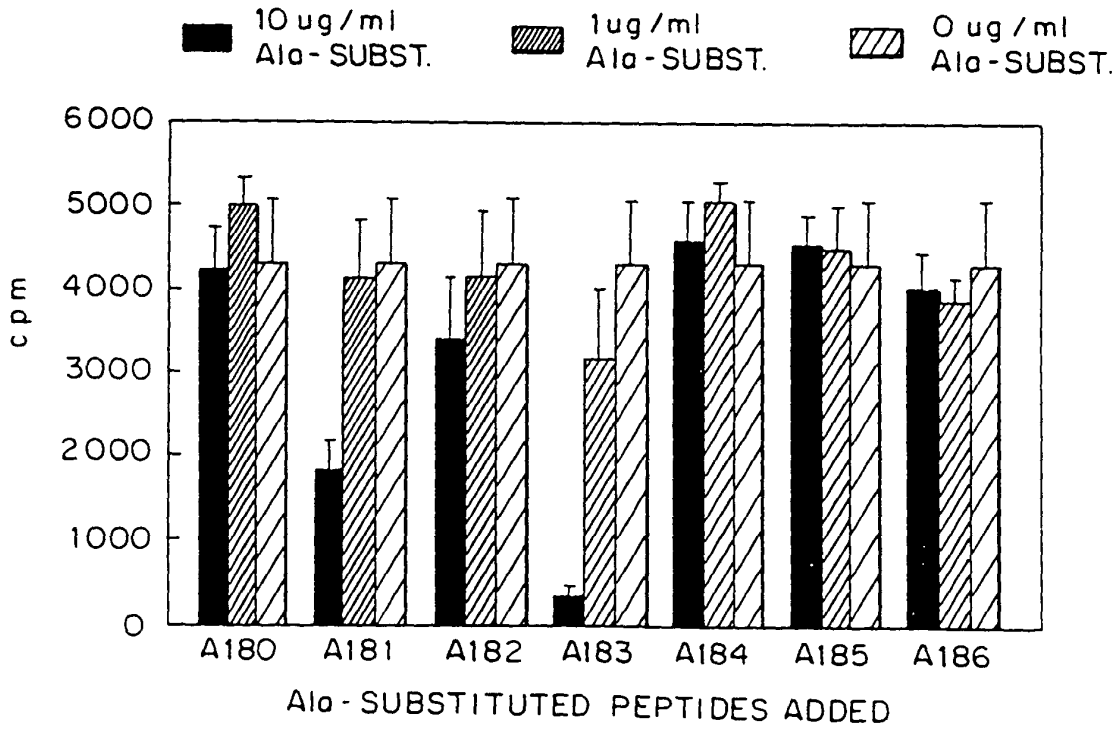


FIG. 3

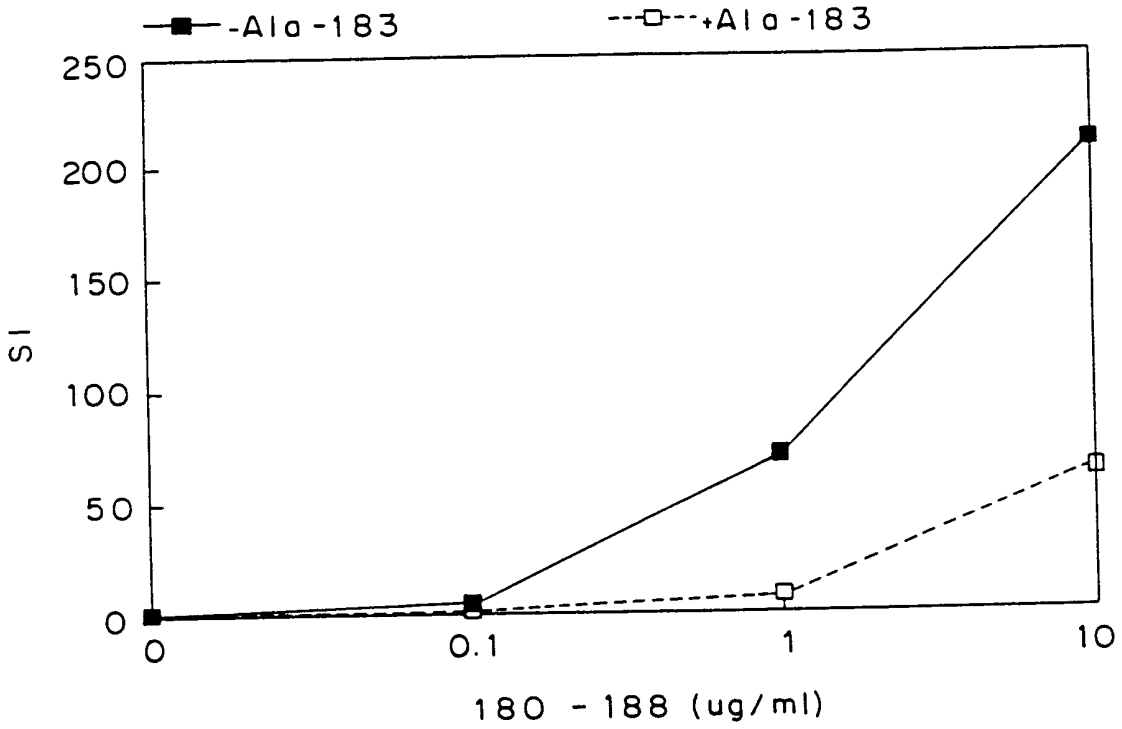
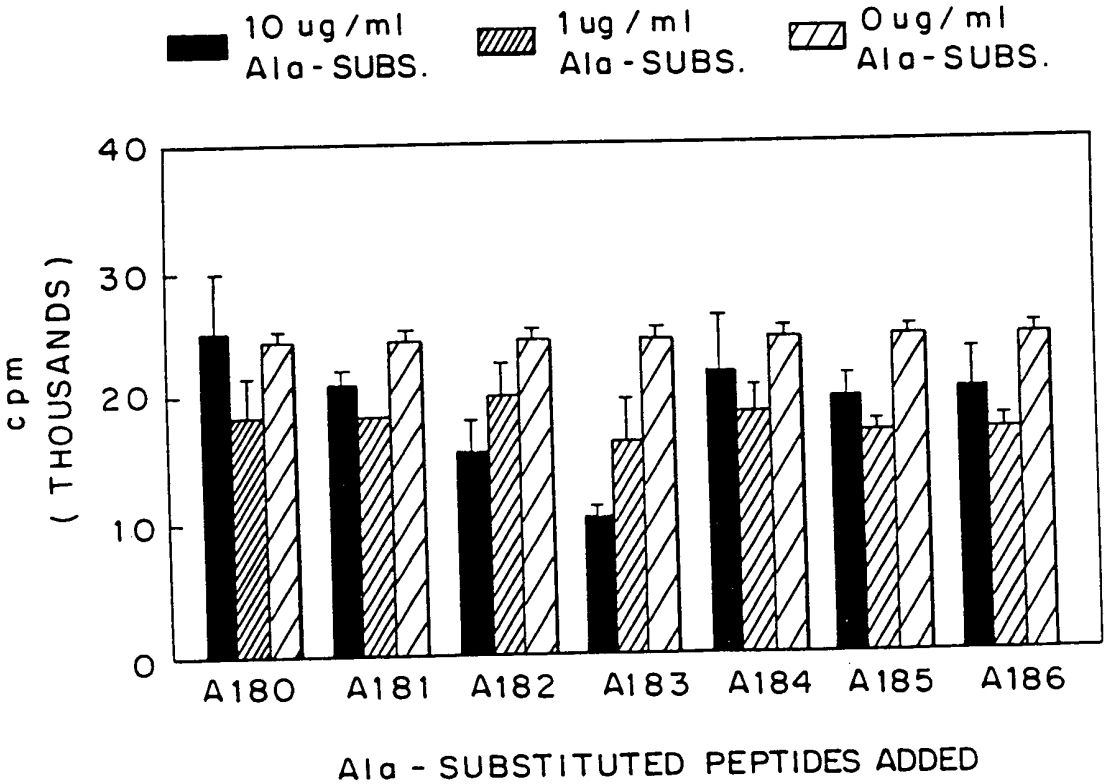

-Ala-183
+Ala-183
250
200
150
100
50
0
SI
0
0.1
1
10
180 - 188 (ug/ml)

FIG. 4


10 ug/ml Ala-SUBS.
1 ug/ml Ala-SUBS.
0 ug/ml Ala-SUBS.
40
30
20
10
0
cpm (THOUSANDS)
A180
A181
A182
A183
A184
A185
A186
Ala-SUBSTITUTED PEPTIDES ADDED

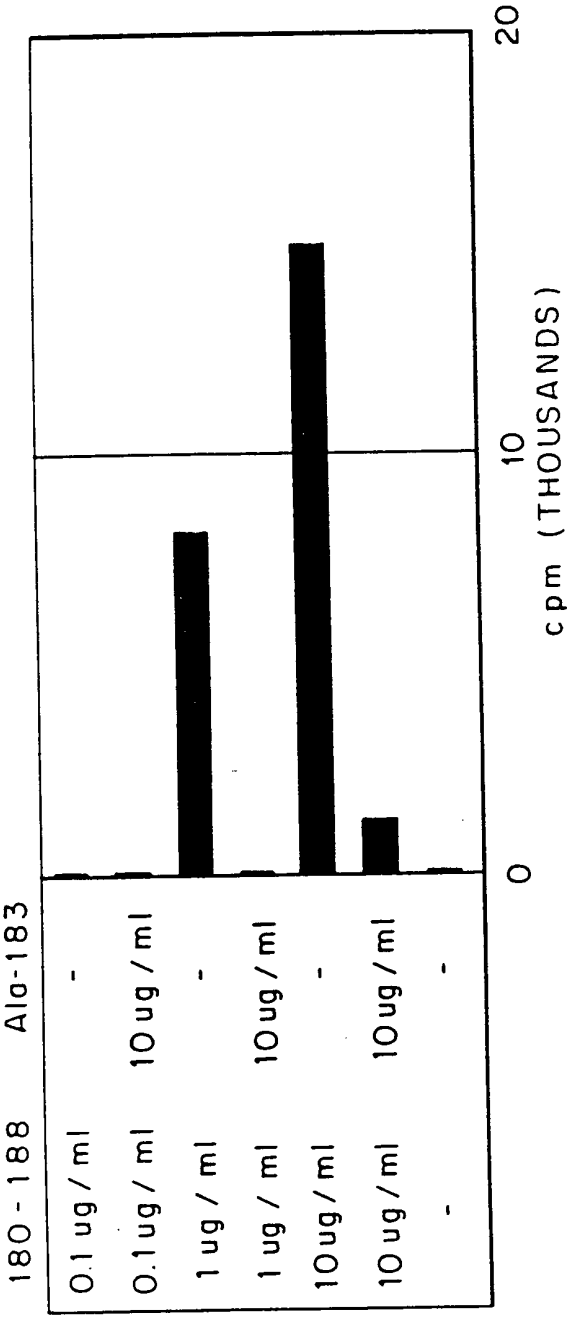


FIG. 5A(a)

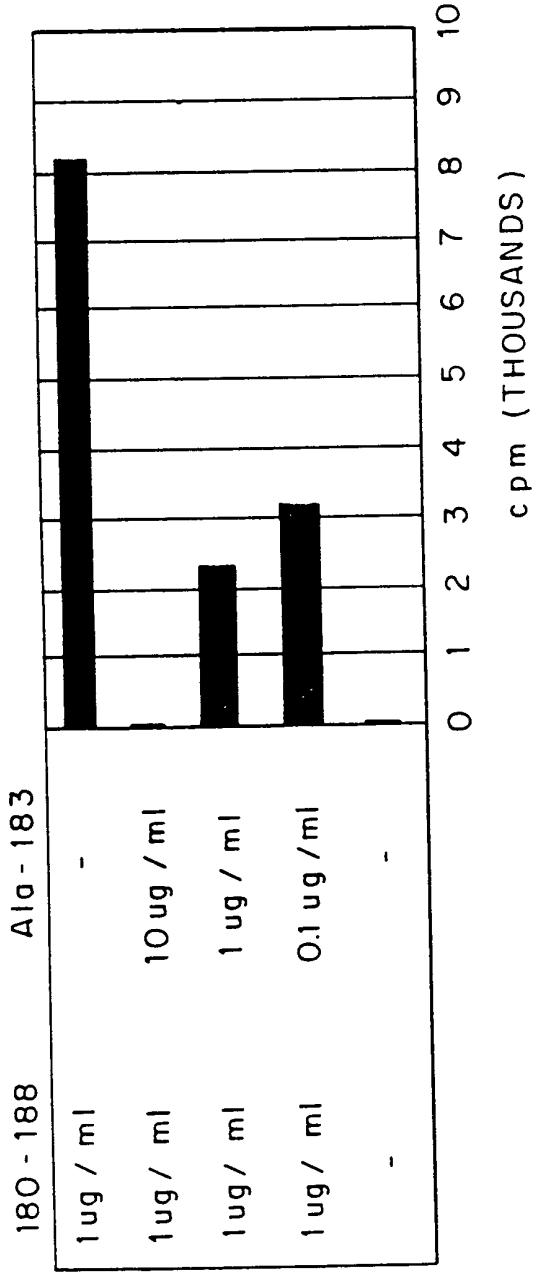


FIG. 5A(b)

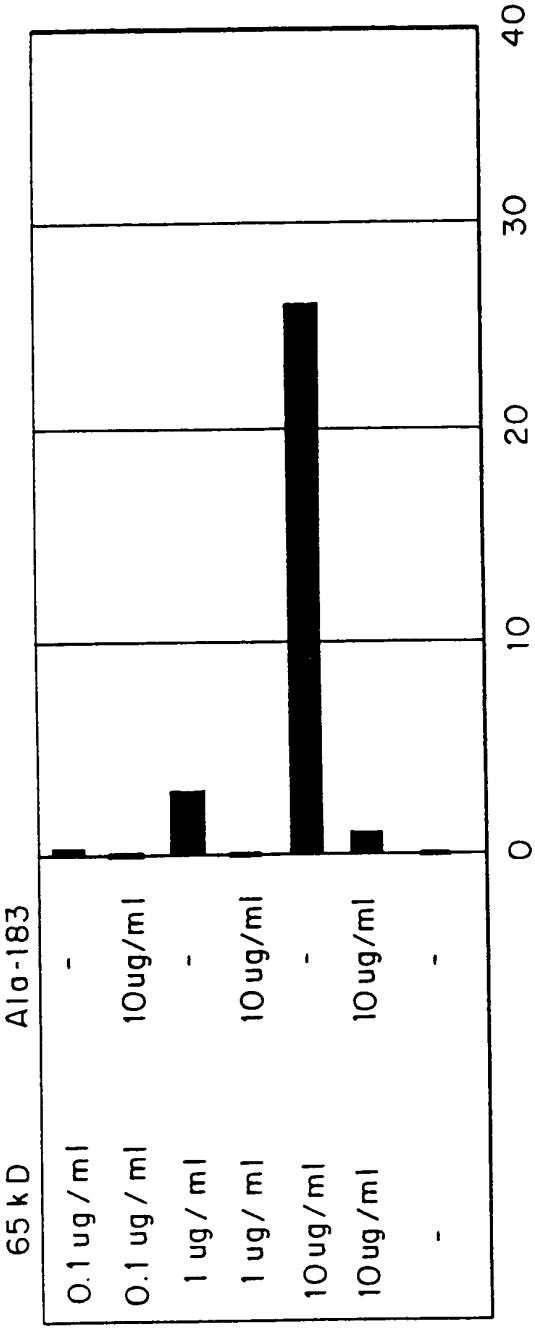


FIG. 5B(a)

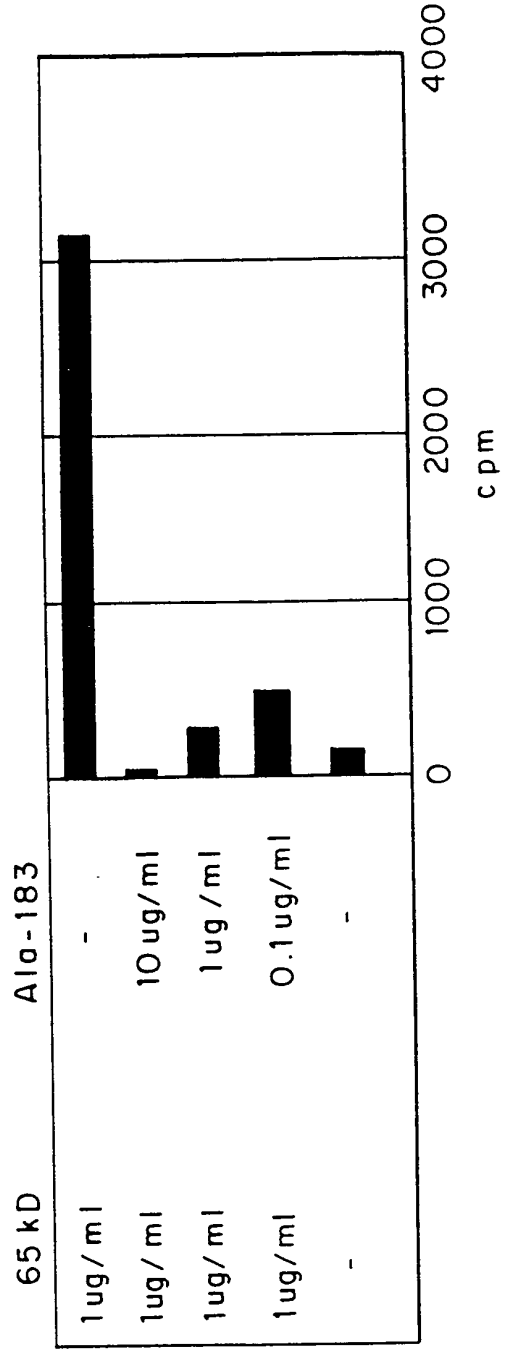


FIG. 5B(b)

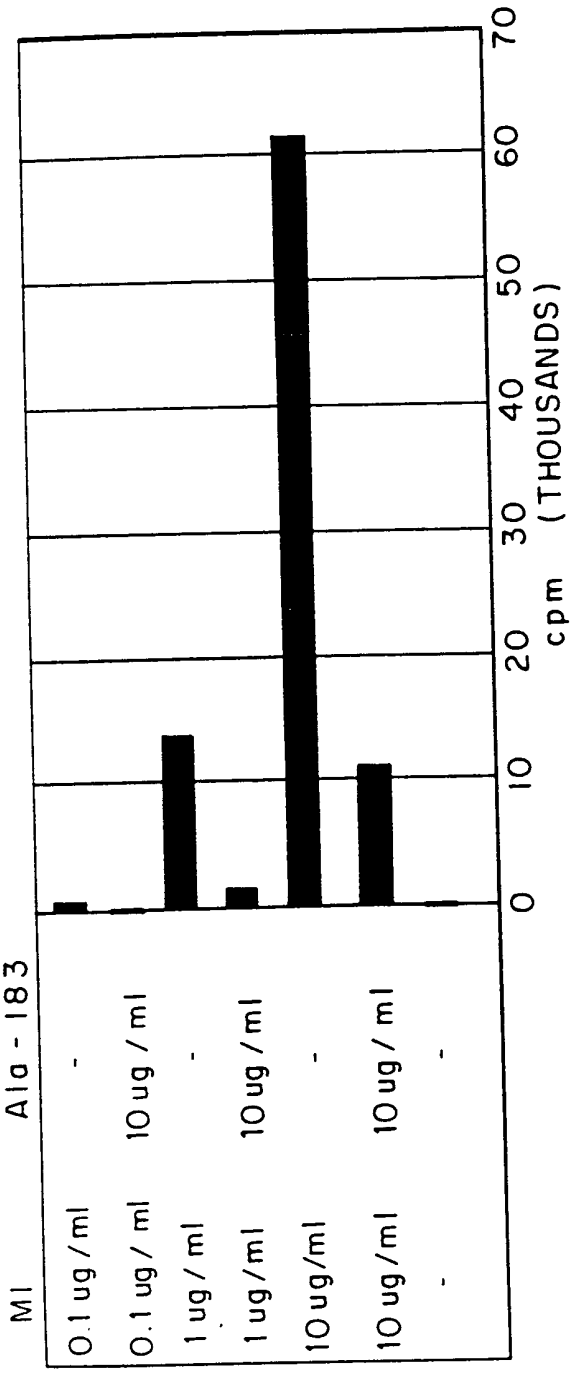


FIG. 5C(a)

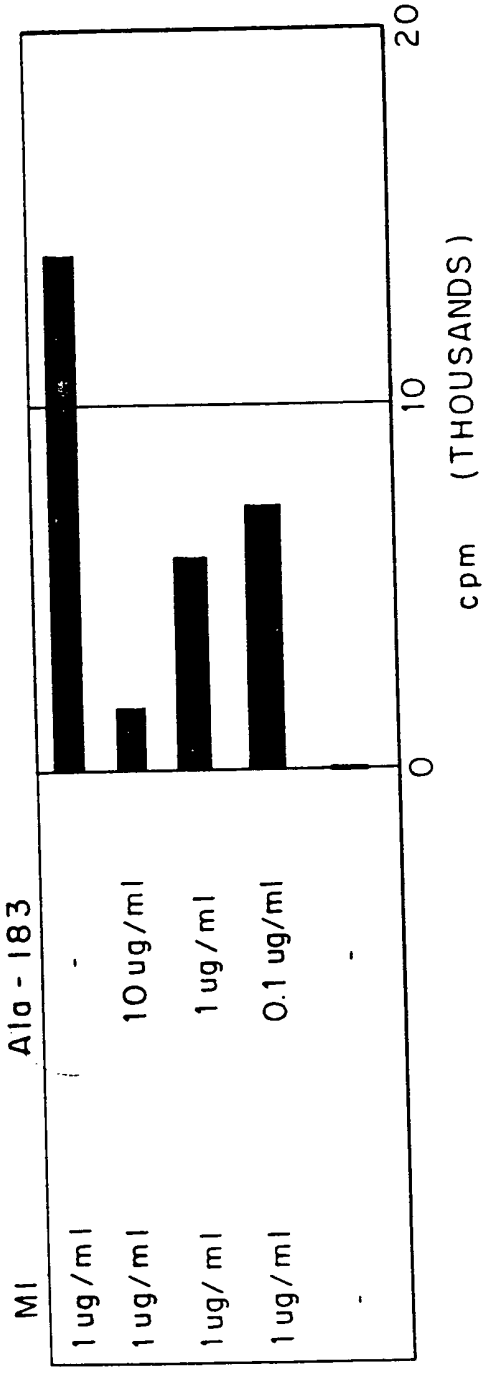


FIG. 5C(b)

FIG. 6

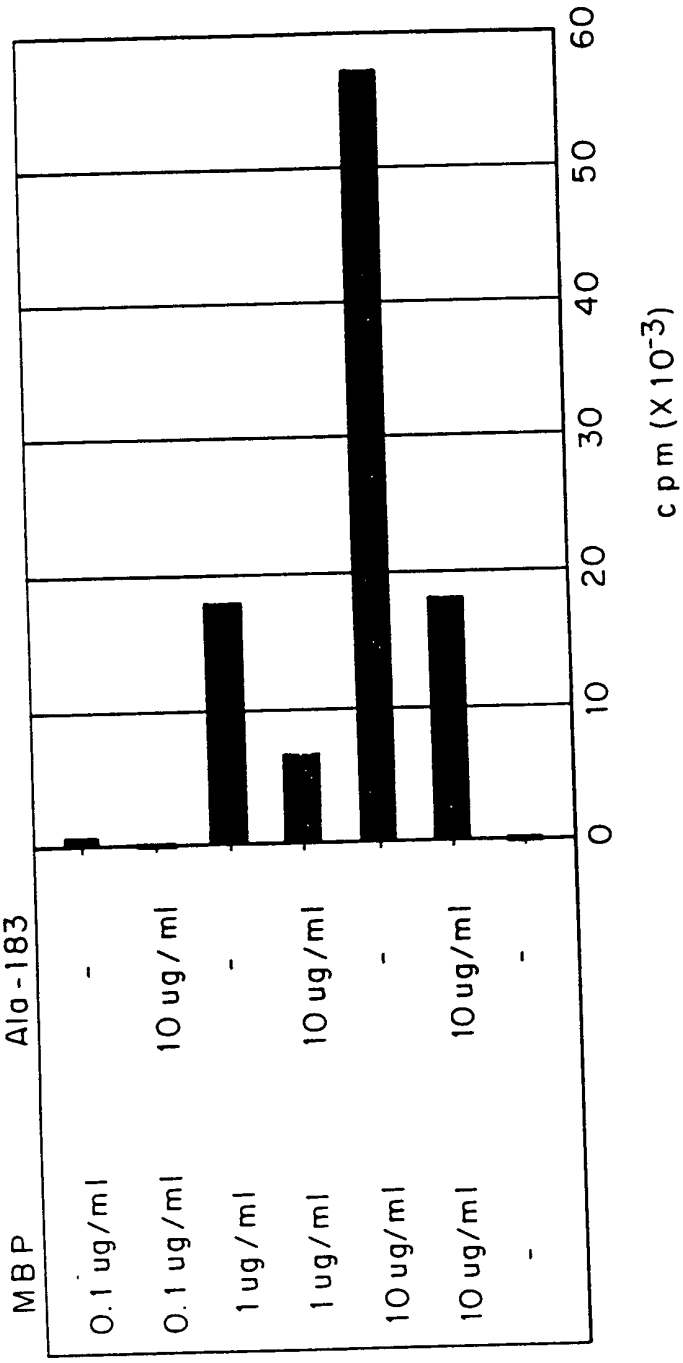

MBP
Ala-183
0.1 ug/ml
-
0.1 ug/ml
10 ug/ml
1 ug/ml
-
1 ug/ml
10 ug/ml
10 ug/ml
-
10 ug/ml
-
0
10
20
30
40
50
60
cpm (X10-3)

FIG. 7

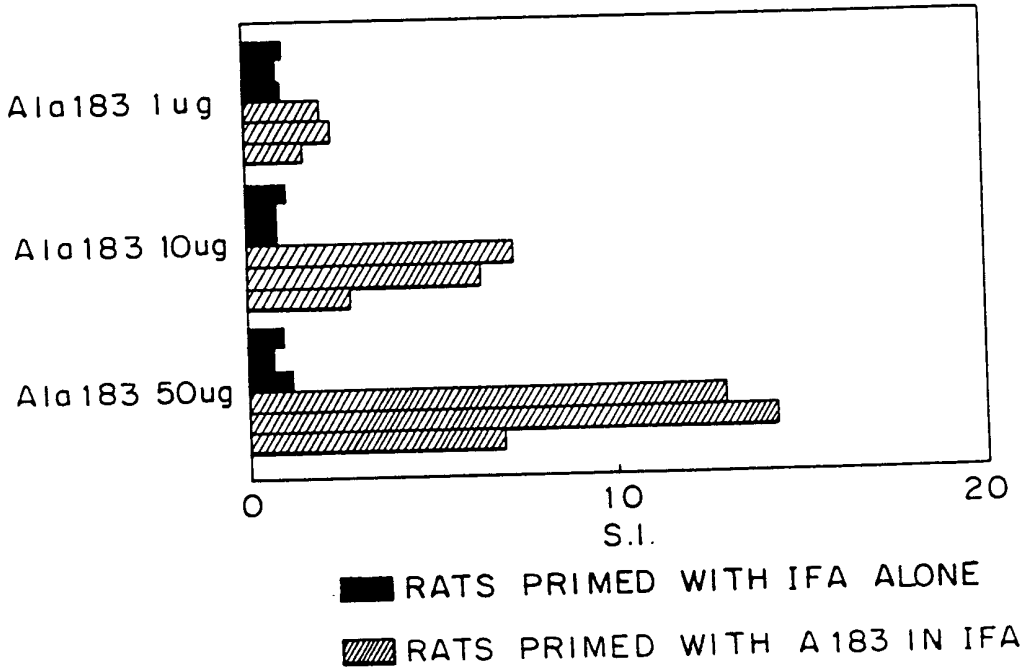

Ala183 1ug
Ala183 10ug
Ala183 50ug
0
10
20
S.I.
RATS PRIMED WITH IFA ALONE
RATS PRIMED WITH A183 IN IFA

FIG. 8

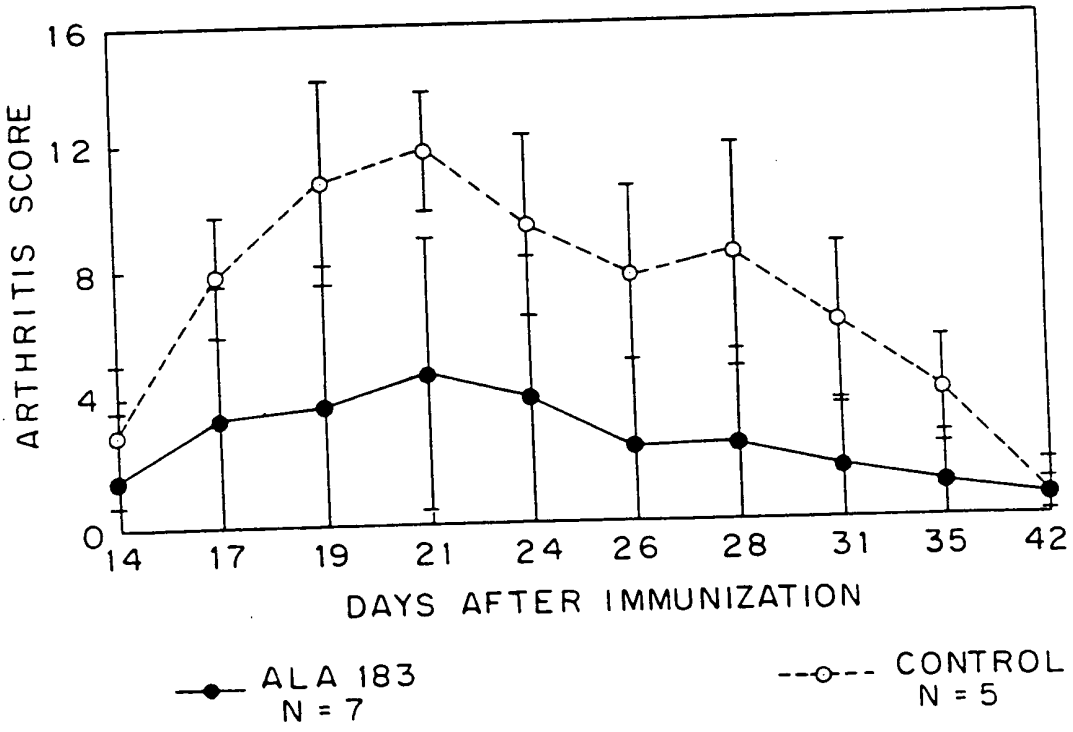

16
12
8
4
0
ARTHRITIS SCORE
14
17
19
21
24
26
28
31
35
42
DAYS AFTER IMMUNIZATION
ALA 183
N = 7
CONTROL
N = 5

FIG. 9A

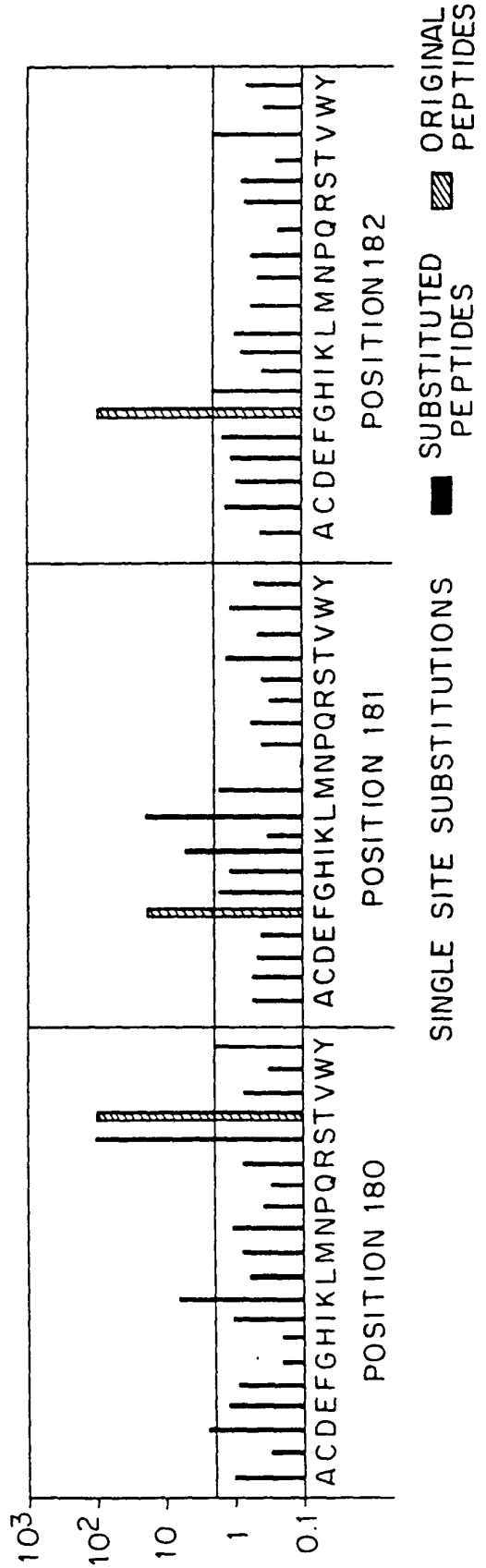

10^3
10^2
10
1
0.1
ACDEFGHIKLMNPQRSTVWY
POSITION 180
ACDEFGHIKLMNPQRSTVWY
POSITION 181
ACDEFGHIKLMNPQRSTVWY
POSITION 182
SINGLE SITE SUBSTITUTIONS
SUBSTITUTED PEPTIDES
ORIGINAL PEPTIDES

FIG. 9B

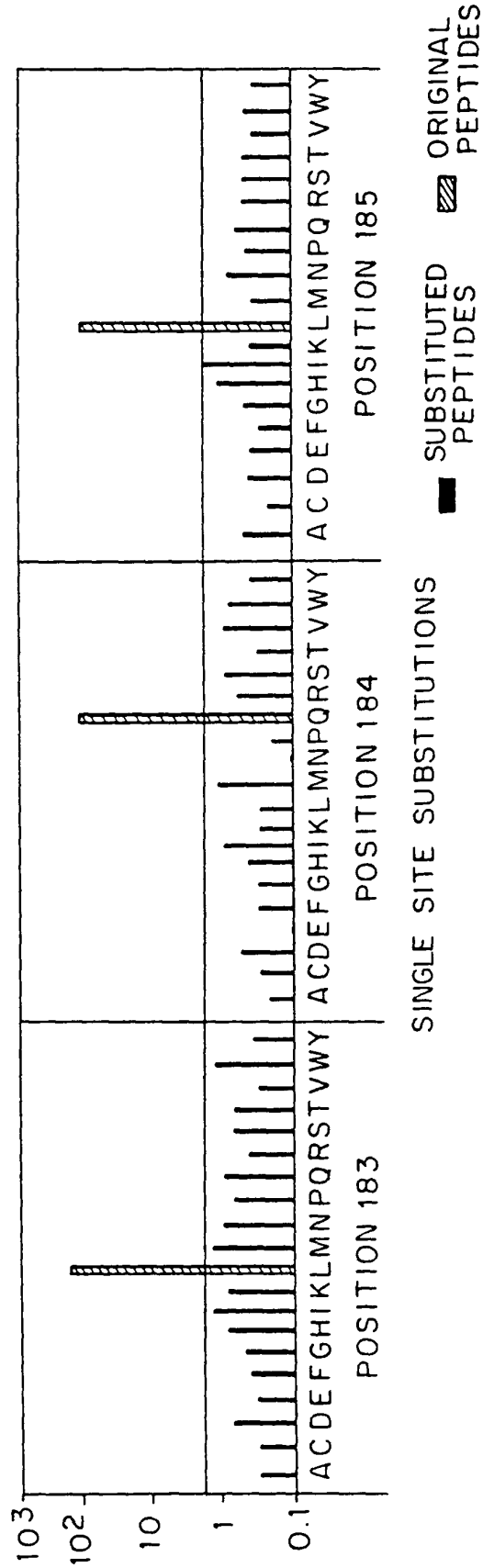
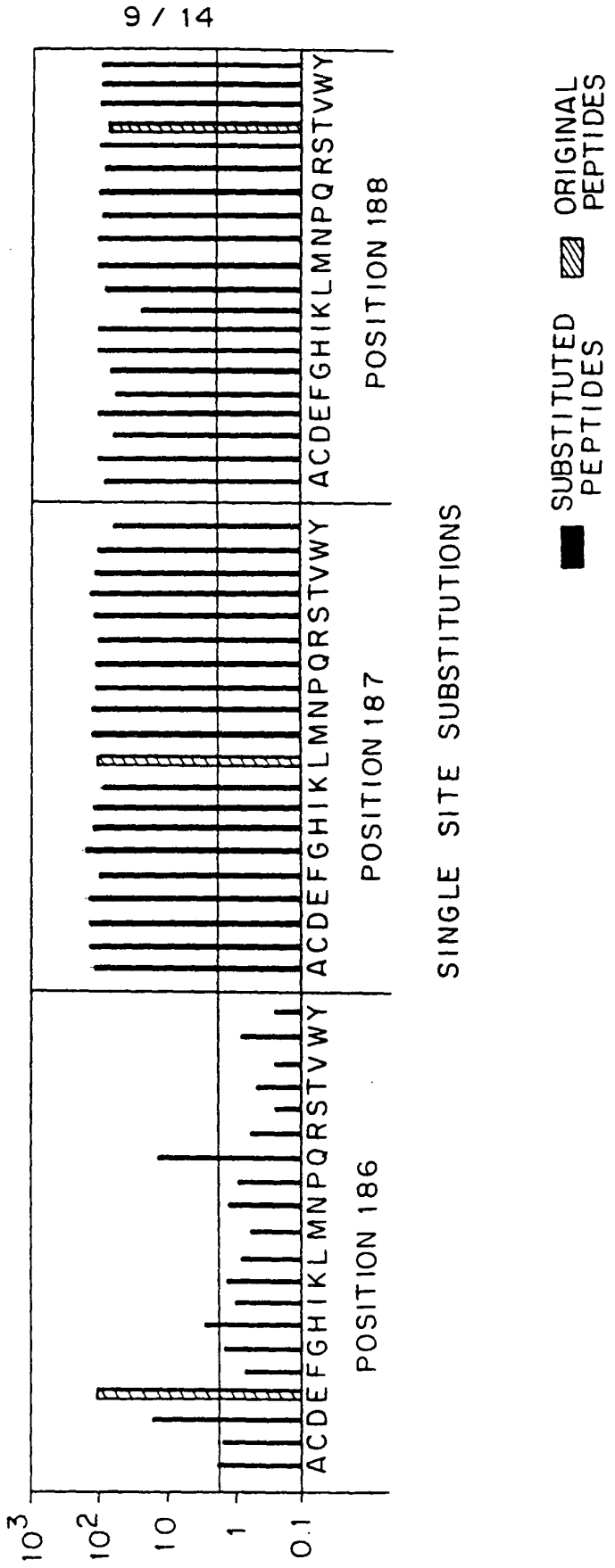

10^3
10^2
10
1
0.1
ACDEFGHIKLMNPQRSTVWY
POSITION 183
ACDEFGHIKLMNPQRSTVWY
POSITION 184
ACDEFGHIKLMNPQRSTVWY
POSITION 185
SINGLE SITE SUBSTITUTIONS
SUBSTITUTED PEPTIDES
ORIGINAL PEPTIDES

FIG. 9C


SUBSTITUTED PEPTIDES
ORIGINAL PEPTIDES
SINGLE SITE SUBSTITUTIONS
POSITION 186
POSITION 187
POSITION 188
ACDEFGHIKLMNPQRSTVWY
10^3
10^2
10
1
0.1

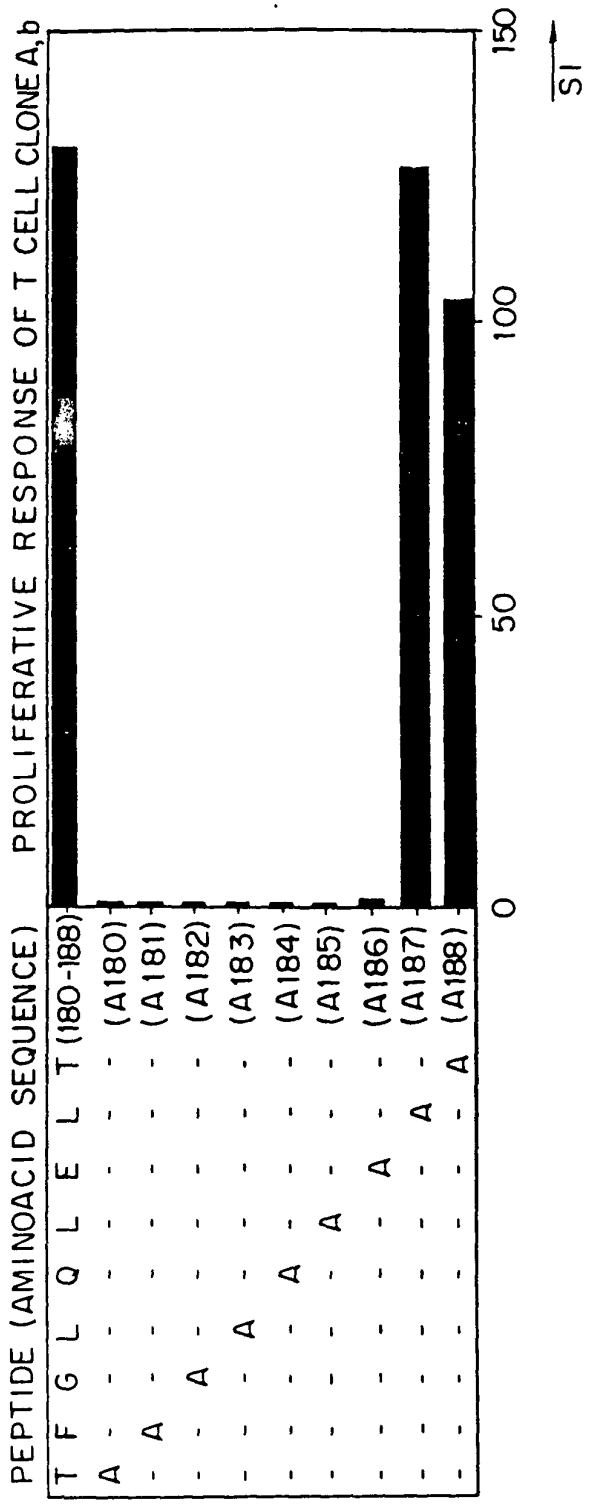

PROLIFERATIVE RESPONSE OF T CELL CLONE A,b
SI
0
50
100
150
PEPTIDE (AMINOACID SEQUENCE)
T F G L Q L E L T (180-188)
A - - - - - - - (A180)
- A - - - - - - (A181)
- - A - - - - - (A182)
- - - A - - - - (A183)
- - - - A - - - (A184)
- - - - - A - - (A185)
- - - - - - A - (A186)
- - - - - - - A (A187)
- - - - - - - A (A188)

FIG. 10

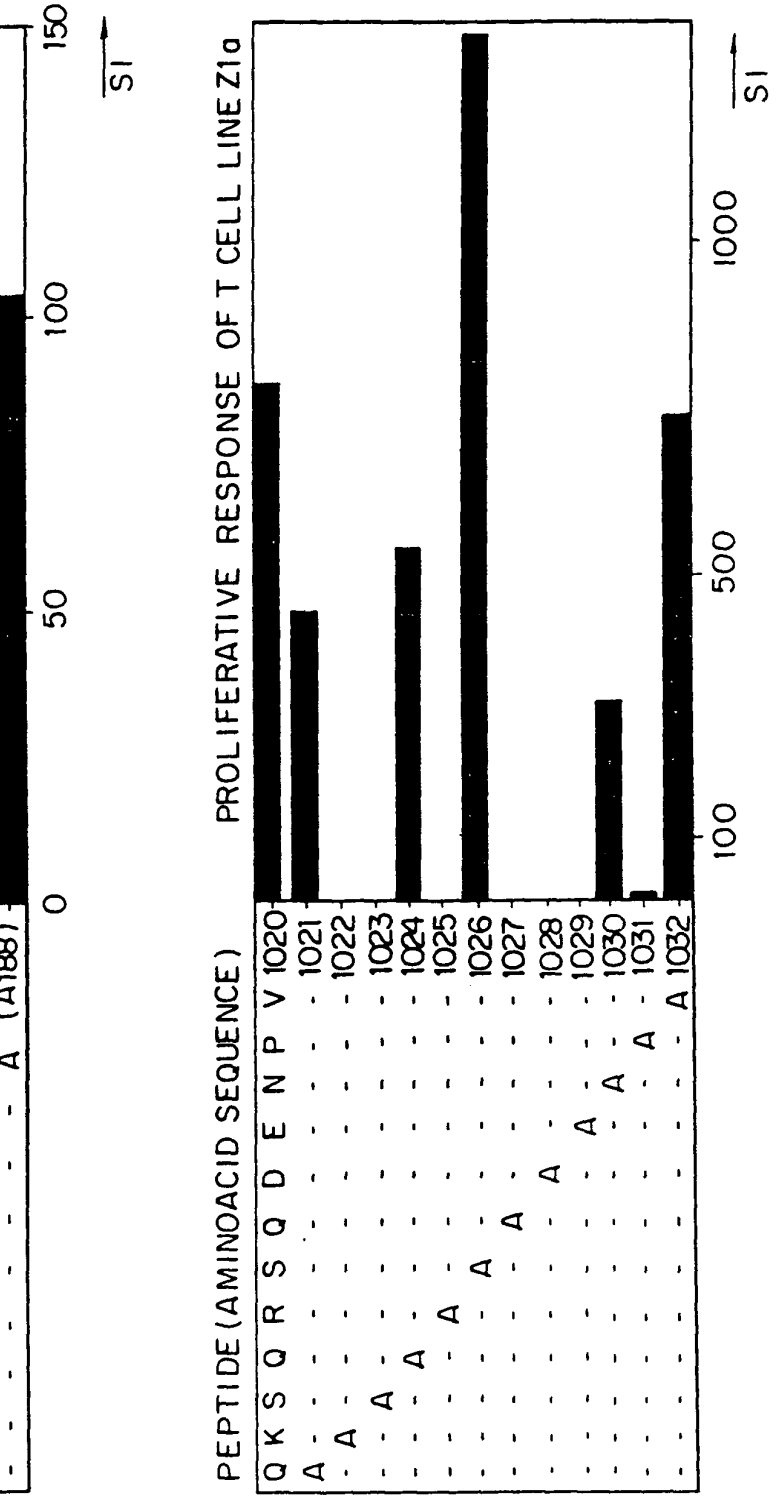

PROLIFERATIVE RESPONSE OF T CELL LINE Z1a
SI
100
500
1000
PEPTIDE (AMINOACID SEQUENCE)
Q K S Q R S Q D E N P V 1020
A - - - - - - - - - - - 1021
- A - - - - - - - - - - 1022
- - A - - - - - - - - - 1023
- - - A - - - - - - - - 1024
- - - - A - - - - - - - 1025
- - - - - A - - - - - - 1026
- - - - - - A - - - - - 1027
- - - - - - - A - - - - 1028
- - - - - - - - A - - - 1029
- - - - - - - - - A - - 1030
- - - - - - - - - - A - 1031
- - - - - - - - - - - A 1032

FIG. 11

FIG. 12

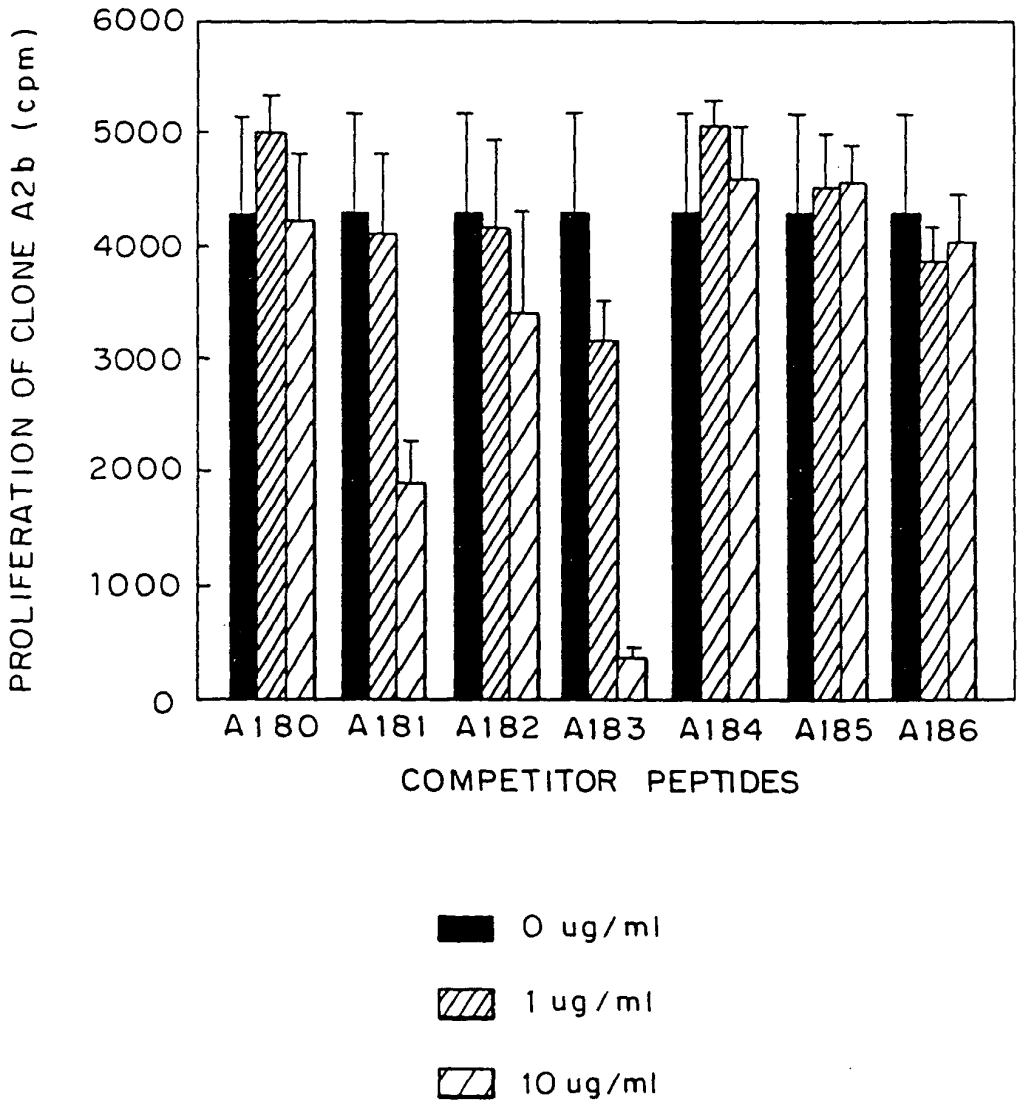

PROLIFERATION OF CLONE A2b (cpm)
6000
5000
4000
3000
2000
1000
0
A180 A181 A182 A183 A184 A185 A186
COMPETITOR PEPTIDES
0 ug/ml
1 ug/ml
10 ug/ml

FIG. 13

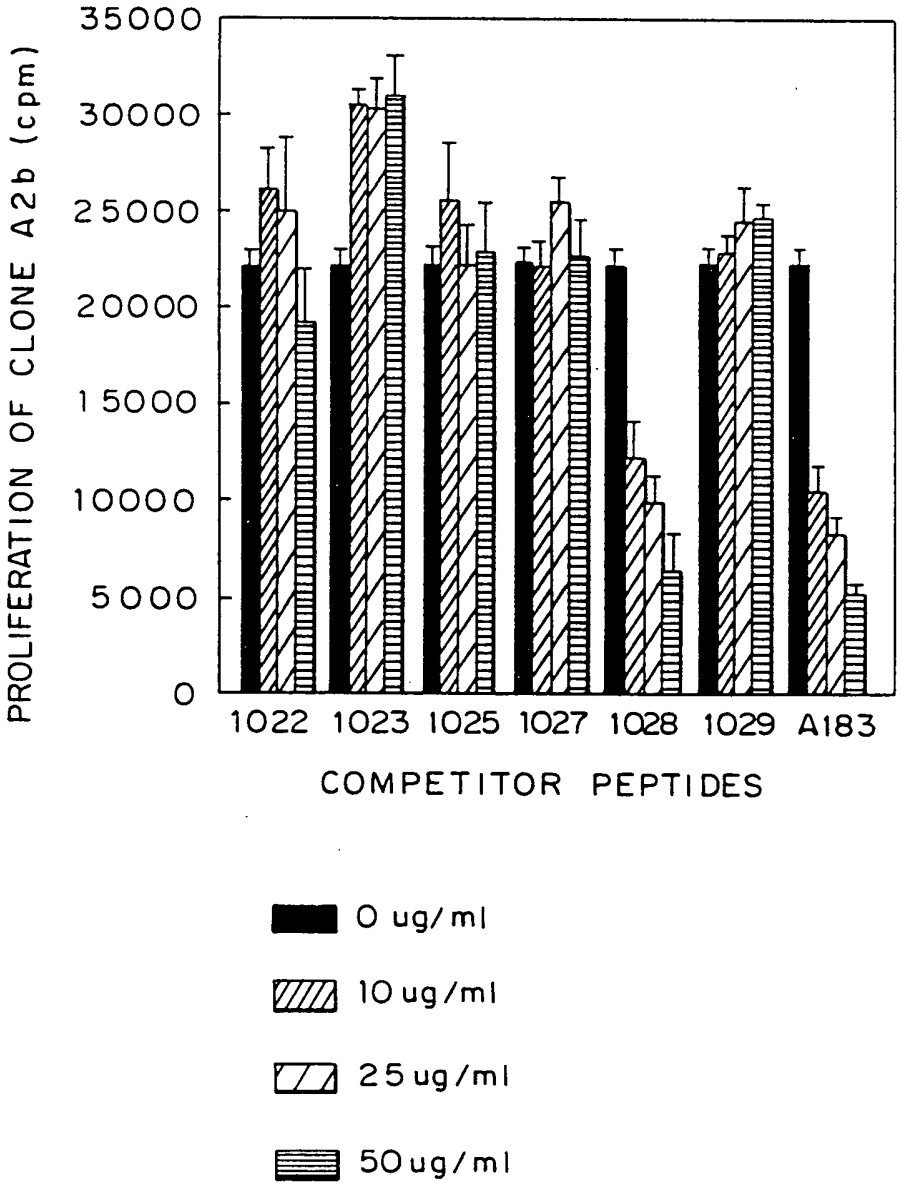

PROLIFERATION OF CLONE A2b (cpm)
35000
30000
25000
20000
15000
10000
5000
0
1022 1023 1025 1027 1028 1029 A183
COMPETITOR PEPTIDES
0 ug/ml
10 ug/ml
25 ug/ml
50 ug/ml

FIG. 14

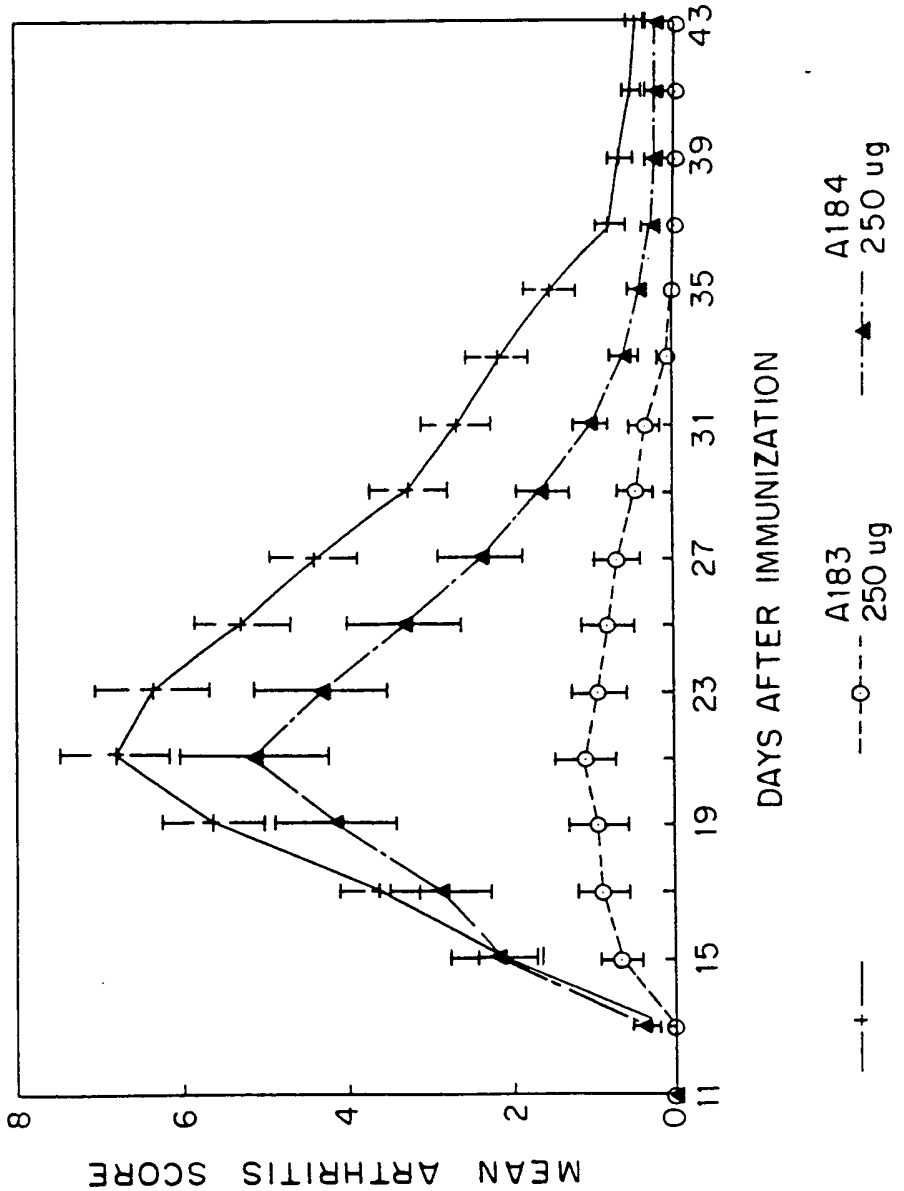
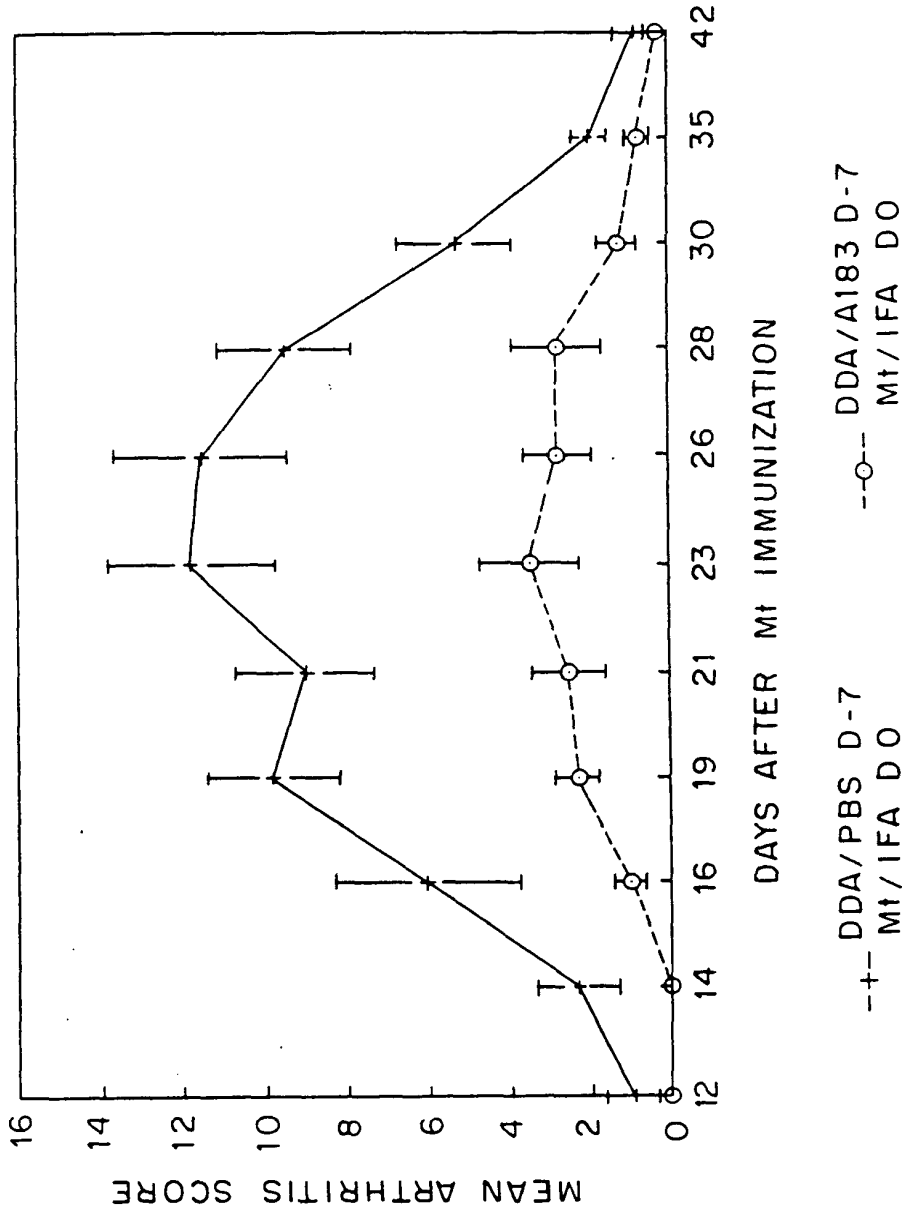

MEAN ARTHRITIS SCORE
0
2
4
6
8
11
15
19
23
27
31
35
39
43
DAYS AFTER IMMUNIZATION
A184
250 ug
A183
250 ug

FIG. 15


MEAN ARTHRITIS SCORE
0
2
4
6
8
10
12
14
16
12
14
16
19
21
23
26
28
30
35
42
DAYS AFTER Mt IMMUNIZATION
DDA/PBS D-7
Mt/IFA DO
DDA/A183 D-7
Mt/IFA DO

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/06434

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 39/00, 37/02; C07K 3/00
U.S. CL.: 424/88, 530/300, 350

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/88; 530/300, 350

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Nature, Volume 331, issued 14 January 1988, vanEden et al., "Cloning of the Mycobacterial epitope recognized by T-lymphocytes in adjuvant arthritis", pages 171-173, see entire article, especially figure 1, page 172.	1-14
X	European Journal of Immunology, Vol. 19, Van de Zee et al., "Efficient mapping and characterization of a T cell epitope by the simultaneous synthesis of multiple peptides", pages 43-47, see entire article.	1-14

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 January 1992	24 JAN 1992
International Searching Authority	**Signature of Authorized Officer**
ISA/US	H. Sidberry ebw

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